

High-Field (9.4T) Magnetic Resonance Imaging in Squirrel Monkey

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INTRODUCTION

The recognition that adult sensory plasticity is both common and robust is one of the most important advances in neuroscience over the past 50 years. Many of the best cellular and systems level studies have explored the mechanisms and limitations of neural reorganization. Unique challenges exist, however, in the study of plasticity. In humans, it is typically impossible to obtain measures of brain organization prior to clinically relevant plasticity-inducing events (for example, stroke, spinal-cord injury or amputation: Cramer et al., 2000; Moore et al., 2000; Staines et al., 2002). Further, a classic paradox in the study of plasticity is that while intervention is necessary to induce reorganization (for example, training, deafferentation, or cortical stimulation) it is also typically required to assess reorganization (for example, craniotomies, injection of anatomical tracers or lesions). While the intent of these studies is to examine plasticity induced by a specific manipulation, the act of probing the system could in some non-linear way combine with the induction process to alter the findings.

The ideal technique for assessing plastic modification of the brain is, therefore, one that is minimally invasive and repeatable, permitting measurement before and after manipulation. Further, this approach should be applicable in non-human primates—respecting the cost and ethical issues that come into play in this regard—and in human subjects, so that parallel studies can be conducted. A technique that permits whole-brain coverage at a relatively fine scale resolution would be an additional benefit, especially for studies of sensory map organization where simultaneous estimation of multiple brain areas can be crucial to a proper understanding of the etiology of neural plasticity.

Non-invasive fMRI is ideally suited for studies of cortical plasticity. In animal model systems, neural organization can be repeatedly assessed following a variety of manipulations (some even performed during a single scanning session), allowing for short and long-term monitoring. Monkey imaging is a particularly powerful tool for studies of plasticity when it is performed using high-field magnets. High-fields deliver the spatial resolution necessary to reveal cortical column-level changes, while still providing whole-brain coverage. Human studies are also commonly conducted with fMRI, allowing direct comparisons between monkeys and humans.

This chapter describes methods we have developed for functional magnetic resonance imaging (fMRI) in squirrel monkeys using a 9.4 Tesla small diameter bore magnet. We begin with a discussion of the strengths and weaknesses of this approach relative to traditional electrophysiology. We then review the methods developed, with an emphasis on the logic driving experimental decisions that have proven critical to the success of imaging experiments. Examples of the anatomical and functional resolution obtained using this paradigm are provided.

Our current discussion is targeted to neuroscientists who may be considering this approach. As such, we attempt to provide a specific and practical discussion of the methods developed, and an introductory background to the factors driving different MR imaging choices.

Classical Sensory Neurophysiology and fMRI: Complimentary Strengths and Weaknesses

A central goal of neurophysiology is to understand how neural activity contributes to sensory perception. To this end, single-neuron electrophysiological techniques, including single

electrode, multi-electrode (stereotrode, tetrode) and intracellular recording approaches are invaluable. These approaches are, however, limited in several ways. Each electrode has a small field of view (FOV) and can only sample the activity of, at most, several neurons. Also, despite many recent advances in implant technology, only ≤ 200 electrodes can be maintained in a single animal. Given the presence of millions of neurons in a single cortical area, this sampling coverage is restrictive. Another problem faced by single neuron recording techniques is that they are invasive. They require surgical intervention, leading to the termination of the animal at the cessation of acute experiments, or to implantation of a systemic alteration in the anatomy and physiology of the animal (e.g., the addition of a novel head apparatus). Further, these techniques are inappropriate for studying human subjects except in the case of brain surgery.

Perhaps the most profound problem with sensory electrophysiology is that an investigator must choose the optimal brain site for electrode placement prior to investigation. In some cases, this assumption can be guided by prior research using alternate methods: In other cases, substantial effort can be exerted to explicate the function of a brain area that is only tangentially related to the execution of a given sensory processing function.

Functional magnetic resonance imaging (fMRI) provides a complimentary set of strengths and weaknesses to those of classic electrophysiology. The weaknesses of fMRI derive from the uncertainties surrounding the origin of the blood flow signal and the resolution of fMRI measurements. The most common method used to measure fMRI activity is the blood-oxygen level dependent signal (BOLD) (Ogawa et al., 1990). Several studies suggest that the BOLD signal is correlated with electrophysiological activity, and that this hemodynamic measure may reflect more subtle, subthreshold potential changes (Logothetis et al., 2001; Heeger et al., 2000; Rees et al., 2000; Backes et al., 2000; Arthurs et al., 2000; Ances et al., 2000). Despite these several correlative findings, the mechanisms linking neural activity and BOLD signals are not fully understood. Further, even if a perfect correlation between net changes in activity level and BOLD can be assumed, this measure is likely 'blind' to changes in temporal patterning that do not require increased neural activity (but see Thompson et al., 2004).

A second weakness is that, relative to electrophysiology measures, the resolution of fMRI is limited in spatial and temporal specificity. The spatial resolution of human fMRI studies is typically on the order of millimeters. The temporal resolution of fMRI is limited due to the slow onset of the BOLD signal, and because sampling intervals (TR) are typically ≥ 1 sec.

Despite these inherent limitations, the complimentary strengths of fMRI are unique, and the technique is ideally positioned for integration with electrophysiological approaches. First, fMRI provides a remarkable field of view: In many experiments, functional activation can be measured across the entire brain. Second, it is not surgically invasive, and does not require the injection of radioisotopes as used in PET imaging. As such, fMRI is safe for repeated use in humans and animals. Third, fMRI offers the best spatial resolution of the non-invasive neuroimaging techniques available, and advances in MR tools (e.g., scanners, coils) and techniques (e.g., scan sequence design, analysis approaches) continue to enhance the precision of this approach.

Monkey fMRI

For several reasons, conducting fMRI in monkeys has the potential to allow for the 'best of both measurement worlds.' Repeated non-invasive imaging can be combined with subsequent

invasive approaches such as electrophysiology and optical imaging, permitting whole brain coverage or, alternatively, high spatial resolution in a region of interest.

Historically, electrophysiology has been conducted in monkey subjects under the assumption that this model provides the most accurate parallel to neural mechanisms employed in the human. This assumption, while logical, has seldom been directly tested using identical measurement techniques in the two species. The use of fMRI in humans and monkeys provides the opportunity for parallel investigation. Using this approach, the areas activated by identical stimuli can be compared, as can aspects of the dynamics of activation in these regions (e.g., adaptation patterns). If parallel activations are observed, then extensive and detailed electrophysiological studies conducted in non-human primates provide the best inferential link currently possible between normal human brain function and the activity of single neurons. Interestingly, using fMRI, differences between species have already been noted in what are believed to be homologous brain areas (Vanduffel et al., 2002).

Monkey fMRI also provides an ideal system for longitudinal monitoring of plastic changes in brain organization. Because fMRI can be used repeatedly within subjects, baseline data can be acquired by imaging for months prior to the introduction of a manipulation. The plasticity induced by this change (or, the lack thereof) can then be tracked systematically and without further damage to the research subject (e.g., Smirnakis et al., 2005).

Importantly, monkey fMRI can also provide a guide for electrophysiological studies. This prior estimation allows a researcher to screen for the brain areas of greatest potential interest, and allows for targeting recordings to precise sub-regions of a given a brain area. As fMRI resolution increases, for example, specific column(s) of interest within a cortical area can be identified (Kim & Duong, 2002; Duong et al., 2001; Duong et al., 2000; Kim et al., 2000; Menon et al., 1997) and measured using electrophysiology.

High-Field Imaging

One advance in technology that has helped overcome some of the concerns regarding fMRI resolution is the increasing use of high magnetic field strengths (e.g., 9.4T). The signal to noise ratio (SNR) of anatomical and functional MRI increases with the static magnetic field strength: Thus, higher spatial sampling is achievable while still maintaining high SNR within each sample volume, improving both anatomical and functional resolution.

Despite these benefits, machines with high-field strengths such as 9.4 T are currently not available for human testing. One reason is that the small diameter of the magnet bore on most high-field machines precludes the use of humans or large primates. Additionally, there are safety issues that arise when imaging at high-fields (Bottomley & Andrew, 1978). For example, human subjects may experience dizziness or other balance-related symptoms (e.g., vertigo, nausea) upon entering or exiting high-field magnets (Schenck et al., 2000), and there is also a risk of peripheral nerve stimulation (Schaefer et al., 2000; Ham et al., 1997). The full scope of these physiological effects has not been determined, therefore restricting high-field imaging to non-human subjects.

Squirrel Monkey Scanning at 9.4 T

To take advantage of the several benefits of monkey MR for sensory neurophysiology, we have developed techniques for imaging squirrel monkeys (SM) at 9.4 T. The squirrel monkey (SM) was selected as our model system for several reasons. First, SM are semi-lissencephalic (Emmers & Alkert, 1963; Benjamin & Welker, 1957; Welker et al., 1957). Their relatively flat cortical surface is ideal for mapping studies using techniques such as optical imaging (Tommerdahl et al., 2002; Chen et al., 2003) and electrophysiology (Sur, 1984), and facilitate the transition from voxel localization in fMRI to these other approaches. Second, a great deal is already known about the cortical organization of SM. Specifically, the representations and receptive field properties in many sensory modalities have been characterized, including tactile (Jain et al., 2001; Merzenich et al., 1987; Sur et al., 1984), visual (Livingstone, 1996), auditory (Cheung et al., 2001), and vestibular (Akbarian et al., 1992; Guldin et al., 1992) cortices. This species has also been used extensively as a model for studies of cortical plasticity (Plautz et al., 2003; Frost et al., 2003; Nudo et al., 2003; Churchill et al., 2001; Xerri et al., 1996; Merzenich et al., 1993; Garraghty & Kaas, 1991), basal ganglia (Flaherty and Graybiel, 1991;1993;1994;1995), and disease (e.g., dystonia and Parkinson's disease: Blake et al., 2002; Rupniak et al., 1992; Boyce et al., 1990).

Several practical considerations also recommend SM use. They habituate well to handling (Abee, 2000) and, relative to macaque monkeys, there is a reduced risk of zoonotic disease transmission to experimenters. Also, because they are bred successfully in captivity, there is greater ease of acquisition. Last, and perhaps most important to our logic in selecting this model system, SM are relatively small. They have a body weight of $\sim 717 \pm 170.4$ g (Gergen & MacLean, 1962), and have a slender maximal body width: As such, SM can fit within the tight space limitations of higher field scanners (e.g., the 11.7 cm gradient-insert diameter of the 9.4 T we currently use). The primary drawback to using SM is that they are not favored for behavioral studies in primates: However, as discussed below, limitations on the tolerance for subject motion at high-fields likely preclude the use of a monkey behavioral preparation with our current methods.

METHODS

Overview of an Experiment

In a typical experiment, monkeys are obtained from our vivarium and transported to the imaging center. In a surgical preparation room on site, anesthesia is induced, the monkey is intubated and catheterized, and subsequently maintained on isoflurane anesthesia for the duration of the experiment. The animal is positioned in an MR-compatible holding device that reduces head movement and secures one of several surface coils on the head. Sensory presentation equipment is positioned (e.g., a tactile stimulator or a visual screen), and the animal placed in the scanner. A series of anatomical and functional images are then acquired over a 3-6 hour period. Following scanning, anesthesia is terminated, and the animal is transferred back to our home facility.

Non-MR Aspects of the Experimental Approach

Transport

The monkeys are kept in a temperature (20 – 23°C), humidity (30 - 70%), and photoperiod (12 h dark/12 h light) controlled environment where they are single-housed in standard cages with a variety of perches and enrichment devices. The evening prior to an experiment, animals are

placed on overnight food restriction. Animals are transferred to off-site experiments in a customized transport box (PrimacARRIER, by Primate Products) in a climate-controlled vehicle. To minimize the stress incurred by direct handling, animals are trained to enter the box, and the majority of our SM (3 of 4 tested) will enter without further interaction.

Anesthetic Induction

The transport box was customized for these studies. The front opening of the box has a sliding plastic door, through which the animal climbs when being collected for transport, and the two longer side panels have an array of openings for ventilation and observation. Two modifications were made. First, a clear, acrylic panel was inserted and secured against one interior side of the box. A pair of removable handles can be attached to receptacles built onto this panel. These handles pass through the holes in the outer wall of the box and enable one to push the panel to the opposite interior side of the box, creating a squeeze apparatus for anesthetic injection. Second, additional openings were added to the side of the box opposite the acrylic panel, to improve access to the lower limb for the initial intramuscular (IM) anesthetic injection.

Using the squeeze-box apparatus, initial sedation is achieved with Telazol delivered IM to a thigh muscle. Active components of Telazol include tiletamine, a dissociative anesthetic that blocks NMDA receptors (Fish, 1997), and zolazepam, a benzodiazepine tranquilizer that potentiates GABA receptors (Reves & Glass, 1990). The dosages used (6.7 – 8.8 mg/kg) are similar to those recommended for use in dogs: For a ~700 g squirrel monkey, 7.1 mg/kg (0.05 ml) of Telazol provides sufficient sedation (~30 min) to intubate and prepare the intravenous (IV) catheter. In several experiments, we have observed that higher concentrations of Telazol, or repeated injections of low doses, compromise the measurement of stimulus-evoked BOLD signals and delay anesthetic recovery after isoflurane is terminated. Though we have not tested other drugs or procedures for induction, a similar drug, ketamine, is often used in SM (Greenstein, 1975) and, if used at low-levels in macaques (1-2 mg/kg), preserves the fMRI BOLD signal (Leopold et al., 2002).

Atropine sulfate (0.04 mg/kg IM) is administered with the initial Telazol injection (same syringe). The anti-cholinergic action of atropine reduces respiratory secretions, keeping airways clear. Once the animal is positioned in the magnet, atropine is delivered through the IV catheter every 45-60 minutes. When access to the IV line is not an option, (e.g., during long anatomical sequences), glycopyrrolate is administered IM (0.01 mg/kg) prior to the scan. The anti-cholinergic action of glycopyrrolate has a longer acting duration (2-3 hours) than atropine.

Intubation and Catheterization

Endotracheal tubes (ET) used with SM must have an appropriately small diameter, smaller than those used for pediatric purposes. We employ customized, re-usable silicone cuffed 2.5 mm (inner diameter) ETs without wire reinforcement (Med-Caire, Vernon, CT). The length of each tube is customized to fit each monkey such that it spans the distance from the mouth to the manubrium sternum (6.7 cm and 8.0 cm for two monkeys whose data is presented in this Chapter). The patency of the ET cuff is tested prior to intubation by inflating with a syringe. Two to three minutes prior to intubation, a single spray of the topical anesthetic, Cetacaine, is delivered to the glottis to reduce the incidence of laryngospasm. The ET is coated with Lidocaine Hydrochloride Oral Topical Solution and inserted into the trachea using a stylet and laryngoscope (size 1 Macintosh blade). Successful ET placement is determined by observing motion of hairs held at the opening of the ET, condensation on a mirror, and/or

expansion/contraction of a latex covering placed at the end of the ET tube. The cuff is then inflated with air (~2 - 2.5 ml). The ET is secured by way of a velcro strap customized with an opening that fits around the ET connector (15 mm) and wraps around the head.

An IV catheter is then placed in the lateral or medial tarsal, metatarsal or saphenous vein, or alternatively in the lateral tail vein (Brady, 2000) using a 24G x 3/4" Surflo catheter with a 27G needle and Surflo injection plug (Terumo Medical Corporation). A small splint is typically used to prevent the ankle from rotating and corrupting the catheter. To mitigate venous injury, catheter placement alternates between the left or right lower limb, and occasionally the tail, across experiments. Currently, this IV line is used to deliver Lactated Ringers solution throughout scanning (7.5 ml/kg/hr), atropine at 45-60 minute intervals, and slow injections of dextrose following scanning (100-250 mg/kg IV; 5% dextrose in water as a single ~ 3.0 ml slow dosage). In experiments now under way, the IV permits the infusion of contrast agents (e.g., MION see discussion) prior to or during imaging.

Anesthesia, Respiration Rate and CO₂-Level Maintenance

Following ET and catheter placement, the monkey is transported to the shielded imaging room and placed on mechanical ventilation (SAR-830 Series Small Animal Ventilator, CWE, Inc.). Anesthesia is maintained via isoflurane in balance oxygen (0.5 - 0.6L O₂/min). During animal positioning (ear bar insertion, head restraint, placement in cradle), isoflurane is maintained at 1.5% and is subsequently reduced in three incremental steps over ~30 minutes to achieve ~0.5 - 0.6% expired isoflurane (measured with a V9004 Capnograph Series with inspired/expired anesthetic gas, Surgivet). Ventilation is maintained at a rate between 34 - 39 breaths per minute with an inspiration time of 0.5 s and expiration duration of ~1.1 s. The inspired/expired duration ratio of the SAR-830 may be adjusted, an important feature to counteract the effects of positive pressure ventilation. Such ventilation may impede venous return via changes in pleural pressure during the inspiration phase; reducing the inspiration duration relative to expiration time blunts the negative effects of positive pressure ventilation.

Isoflurane is a recommended anesthetic for use in SM (Brady, 2000), and for maintaining stimulus-evoked fMRI signals (Nair & Duong, 2004; Sicard & Duong, 2005; Liu et al., 2004). Isoflurane is a volatile anesthetic that causes a dose-dependent decrease in blood pressure through vasodilation, though the effect of increasing cerebral blood flow is less than that caused by halothane (Reinstrup et al., 1995). The molecular mechanisms of isoflurane action are not completely understood, but it is considered to act on multiple neural membrane proteins, including GABA A chloride channels. Isoflurane has a wide safety margin, analgesic properties, and is associated with a relatively rapid recovery. Importantly, this anesthetic is sufficient to induce muscle relaxation in SM, and muscle relaxants such as mivacurium, often used when imaging larger monkeys (Logothetis et al., 1999), are not necessary for our research.

At the end of the experiment, SM awaken quickly after low level isoflurane is discontinued (~5 - 15 minutes). Therefore, it is important to maintain anesthesia while removing items that could potentially damage an awakening monkey (ear bars, head restraints, surface coils). This transient consciousness is followed by ~2 hours of recovery and the monkey is kept warm using a hot water blanket or rechargeable hot packs during transport. Pulse oximetry is monitored during recovery and fluids (Lactated Ringer's) are delivered IV.

The use of anesthesia is essential for collecting high-resolution primate data in our paradigm. In our preparation, and at the resolution employed, the motion generated in the anesthetized animal is already in some cases in excess of required tolerances (e.g., see Figure 2). The motion observed by an awake, head restrained monkey will almost certainly exceed the spatial resolution at 9.4T and create signal artifacts that are further enhanced because of the greater susceptibility at higher fields. There are nevertheless clear drawbacks to the use of anesthesia. First, anesthesia may depress the BOLD signal, requiring averaging across several fMRI runs for a sufficient contrast to noise ratio. Second, fMRI BOLD signals obtained under anesthetic can be difficult to interpret, as isoflurane has a direct impact on central neural processing and substantial vasodilatory properties (Warltier & Pagel, 1992). Further studies directed to examine the impact of anesthesia on BOLD signals and central nervous system function are essential (Disbrow et al., 2000; Ishizawa et al., 2005). However, preliminary data suggest that the increased SNR afforded by higher field strength may recoup some of the anesthetic induced suppression of the BOLD signal (see Figure 13B).

Physiological Monitoring

Proper physiological monitoring is particularly important where narrow, long bores prevent visual inspection of the animal. Variables we typically measure include: end-tidal CO₂, expired/inspired isoflurane concentration (V9004 Capnograph Series, Surgivet), non-invasive blood pressure from the femoral artery measured between EPI scans (V6004 Series Non-Invasive Blood Pressure, Surgivet), heart rate and arterial oxygen saturation via a pulse oximetry sensor secured to the palm (Nonin 8600V), and rectal temperature. Published normative physiological data for anesthetized SM is scarce, and for this reason Table I lists the physiological data (mean, standard deviation) recorded over five typical scan sessions. In the awake restrained SM, body temperature is 37 - 39°C (Brady, 2000; Pinneo, 1968), mean arterial blood pressure is 140 ± 4 mmHg (Byrd & Gonzalez, 1981), and the heart rate may exceed 300 beats per minute (Pinneo, 1968). As Table 1 indicates, isoflurane anesthesia decreases body temperature, blood pressure and heart rate. Stimulus-evoked activation is robust in sessions when end-tidal CO₂ levels range between 35 - 40 mm Hg; sessions with lower capnic levels require greater signal averaging to reveal significant activation.

Monitoring animal physiology in small bore magnets is challenging. One challenge is to locate devices that are MR compatible at high-field strengths: In our experience, devices advertised as 'MR compatible' can have components that are readily corrupted at 9.4 T. Also, as mentioned above, there is virtually no visual information available to indicate the health condition of the monkey, making physiological monitoring across several variables a necessity. Another practical consideration is that input/output connections (e.g. the pulse oximeter cable) should avoid coursing beneath the surface coil (Figure 1).

One benefit of fMRI is that it can be repeatedly conducted in the same subject (animal or human). Nevertheless, the anesthetized preparation described above involves repeated procedures that could impact the health of the animal. These include the increased risk of infection associated with repeated intubation, anesthesia and IV, a risk of sensory damage to repeated placement of the animal in the noise environment of the scanner, and potential adaptation to anesthetic state. Thus far, we have observed that the above procedures promote a healthy recovery and allow longevity of the animals in experiments: The fMRI data and most anatomical images reported here are from two monkeys scanned repeatedly (~twice per month) over the course of 1 year. In two cases where significant complications were observed,

monkeys had not been sufficiently acclimated to the anesthetic treatment and/or to travel to the scanning facility: These steps are now standard procedures in our design.

Body Positioning and Head Stabilization During Scanning

Following intubation, catheter placement, and ~10 minutes of isoflurane, muscle tone is sufficiently low and the monkey is placed in the custom-made cradle shown in Figure 1. The length of the cradle encases the entire elongated outstretched body, including the tail. The body of the cradle is made from plastic piping (ID 9.5 cm, OD 11 cm), and the outer surface is covered in rubber foam pipe insulation tape (~1 mm thick) to dampen the transfer of magnet vibrations to the preparation, and to provide frictional resistance to micro-motions of the apparatus. Placement of SM in the cradle is in the prone position, atop a heated water blanket (Gaymar Therma Pump, Harvard Apparatus). The body is extended with the arms outstretched in front of the animal for presentation of tactile stimuli.

One advantage of using an anesthetized preparation is that subject motion is minimized. However, the resolution of EPI images (e.g., 625 μm in-plane) and typical anatomical images (e.g., 195 μm) demands minimal motion, and motion-related signal artifacts are amplified at high-fields. Therefore, proper head restraint is mandatory for successful experiments. We have found that a triangulation of restraint—chin rest, ear bars and head piece—is necessary to reduce subject motion to an acceptable level. Examples of subject motion in a functional scan taken with and without this triangulation are shown in Figure 2. With each restraint in place, subject motions of less than ~190 μm are routinely observed across a ≥ 3 hour scanning period. Because subject motion poses a substantial challenge at the resolution we employ, each functional scan is subjected to motion assessment immediately following its acquisition (*AFNI*: Analysis of Functional Neuroimages). The on-line motion estimation provides a rapid assessment of the effectiveness of the head restraint and anesthesia depth, either of which can be subsequently adjusted.

Using three points of restraint is key to the precise repetition of the head position across experiments and to the reduction of head motion within experiments. These points are the *chin rest*, *ear bars* and *head piece*. The chin rest consists of a hard rubber stopper (2.4 cm height) secured on the bottom of the cradle. This piece prevents downward motions of the head, ensures accuracy of the height and angle of head placement, and helps prevent the ET from being inadvertently dislodged or compressed (see Figure 8A). The ear bars are cylinders (3.7 cm length x 0.4 mm diameter) tapered at their insertion tip to be non-rupturing. They are positioned at a height of 4.2 cm from the floor of the cradle. The ear bars are held in a 0.5 mm slot with a tapped opening on the posterior side for a delrin plastic thumb screw. Prior to insertion into the ears, the bars are coated with topical anesthetic (Lidocaine HCL Jelly, 2%, Teva). The head piece, as shown in Figure 8C, consists of a horizontal rubber slab joined at 2 points to a ‘Y’ support. The anterior-posterior position of the Y-piece is adjusted to compensate for the head dimensions of each monkey. To achieve identical cradle placement across scan sessions, a peg is inserted through an opening in the posterior base of the cradle into a hole in the gradient coil insert.

Tactile Sensory Stimulation

We have conducted successful tactile and visual BOLD fMRI of SM in the 9.4 T scanner: Because the focus of our studies thus far has been on the tactile, we describe our approach in that modality here. In tactile experiments, accurate between-session hand placement and consistent

site of stimulus delivery within a session are essential. To provide stability, the left hand of the monkey is fitted into a custom rubber mold made from a double casting of the monkey's hand (Mix-a-Mold, AMACO, Indianapolis, IN), the positive is then cast into a rubber mold (PMC-121, Smooth-on, Inc., Easton, PA). This mold regularizes hand placement, separates the fingers and damps non-specific vibration transmission. The hand mold is mounted to an L-shaped acetyl plastic housing (2.0 cm thickness) that also secures the vibrotactile elements. The finger position is maintained via plastic cable ties, and two velcro strips maintain the wrist. Figure 3 shows a schematic of the stimulator. Piezoelectric (PZ) elements (Noliac, Denmark: 3.2 x 0.78 x 0.18 cm) are used to deliver mechanical vibrotactile stimulation to the glabrous surface of the hand. These elements are favored because their multi-layered PZ synthesis provides a high relative force generation and a high fundamental resonance (typically >700 Hz in a fixed-free condition). Stimuli are usually applied to the distal and middle segments of the second digit, though fMRI-compatible stimulators have been made for human and monkey with a greater number of elements (e.g., 9 independent stimulators). Each PZ is equipped with a 3 mm diameter delrin post that vibrates perpendicular to the skin surface through an opening in the mold. A third PZ element secured at a 2.0 cm distance from the hand (not in contact with the skin) has been used to deliver vibration to the device but not directly to the finger, to emulate non-specific effects of PZ activation during control, non-stimulation runs.

Tactile presentation is controlled via custom software developed in MATLAB. Using a portable computer (BSI) with slots for four full sized PCI cards, signals are sent through an array of National Instruments digital output cards connected to a BNC panel. Currently, the system controls up to 16 tactile and 2 audio independent channels, though the software is designed to accommodate additional output. For a typical experiment, a $\leq 10V$ signal is sent through a 15X amplifier (Sensor Technologies) to the PZ. During imaging, timing of stimulus presentation is yoked to data sampling to prevent errors due to drift in scanner timing: The scanner sends a TTL output at the beginning of the scan that is routed through a digital port on the BNC panel. The MATLAB program registers this pulse and triggers the program to output a signal that contains the programmed on/off durations, waveform type, amplitude and frequency.

To calibrate the amplitude of PZ movement, we built an optical sensor system with custom software. This calibration is important for reducing between-session variability in the output of the PZ, as these elements can degrade steadily or suddenly over time. As shown in Figure 4 (left), the optical sensor (Fairchild Semiconductors, QVE11233) is mounted on a micromanipulator, and recordings are made on an immovable steel platform. While the plastic post extension of the PZ is in contact with the monkey skin, the optical sensor registers changes in the displacement of the vibrating probe by detecting motion of a side attachment that breaks the light beam, and changes an input driving voltage. An example of a calibration experiment is shown in Figure 4 (right). The input voltage used in typical tactile experiments, indicated by an asterisk, evokes $\sim 80 \mu\text{m}$ of indentation to the skin surface.

MR Aspects of the Experimental Approach

The MRI system we currently use is a Magnex Scientific 9.4 T 20 cm inner diameter horizontal bore magnet, with a gradient strength of 200 mT/m with fast gradient switching (100 μs rise time). The system is equipped with Bruker Avance console, and has an effective ID of 11.7 cm with the gradient inset. The advantage of higher static field strength (B_0) is increased SNR, due to the greater proportion of proton magnetization, with the net gain of SNR increasing as the

square root of the static magnetic field (Gati et al., 1997). One benefit of greater SNR is the gain in anatomical resolution, which permits the identification of subtle features (e.g., cortical laminae). Similarly, for functional imaging purposes, the contrast to noise ratio of magnetization differences between oxy- and deoxy-hemoglobin is much greater at higher field (Yacoub et al., 2003; Yacoub et al., 2005), enhancing the BOLD signal.

The BOLD signal is the most common contrast agent used to measure functional activity, and there are many excellent reviews that describe what is known of its neural origins (Arthurs & Boniface, 2002; Logothetis & Pfeuffer, 2004; Logothetis & Wandell, 2004). In brief, the BOLD signal depends on blood flow, blood volume and the ratio of deoxygenated to oxygenated hemoglobin. An increase in neural activity evokes a concomitant increase in the BOLD signal due to an increase in the relative concentration of oxygenated hemoglobin that exceeds the local requirement for oxygen.

The BOLD signal can be obtained using *gradient echo* (GRE) or *spin echo* (SE) imaging. In GRE imaging, the BOLD effect is derived from both microvascular (e.g., those that perfuse brain tissue) and macrovascular (e.g., large draining veins) sources, and generally provides greater SNR than SE. In SE imaging, a refocusing pulse reduces the contribution of large blood vessels to the BOLD signal, thereby improving the spatial localization of fMRI activity to the activated neural tissue (Lee et al., 1999; Yacoub et al., 2003; Yacoub et al., 2005). For example, visually evoked signal changes obtained with SE have been localized to the approximate position of layer IV in cat visual cortex (the input layer), while under the same paradigm GRE BOLD signals were observed at the cortical surface (Zhao et al., 2004; see also Yacoub et al., 2005). Because of the increased SNR at high-field, which helps compensate for potential loss of signal when not using GRE, SE imaging provides an excellent opportunity to reveal the spatial specificity of the BOLD signal.

The second reason for choosing SE imaging relates to signal loss at tissue interfaces. Each tissue type (e.g., bone, dura, brain) exhibits its own magnetic properties when subjected to a static magnetic field. The interface at mismatched tissue types creates a local magnetic gradient that results in an inhomogeneous magnetic field, an effect called magnetic susceptibility. This effect is amplified at high-field, and poses a greater challenge to imaging across a large, inhomogeneous sample. The GRE sequence is specifically sensitive to the susceptibility effects, and signal dropout is often seen at locations where mismatched tissue types meet (e.g., near the sinuses and ear canal). Effects of the susceptibility induced signal inhomogeneity on SE and GRE images are shown in Figure 5.

For the reasons highlighted above, our BOLD fMRI sequence is a single-shot SE sequence and is depicted in Figure 6. Using this sequence, the entire spatial frequency domain (k-space) is acquired with a single repetition (90° RF, 180° RF pair), requiring only ~40 ms per 2-D image set. However, as is common in echo-planar imaging, in exchange for rapid data acquisition, images suffer from geometric image distortions. The fMRI acquisition can stretch or compress images when compared to the non-distorted anatomical images. These geometric distortions may be caused by magnetic susceptibility and are particularly severe at high-field strengths. Distortion reduction requires improvement in the magnetic homogeneity over the sample volume by optimizing electric currents in shim coils. Although we had some success with automated shimming routines (e.g., FASTMAP; Greuetter & Tkac, 2000), the improvements were modest, and we found that manual shimming improved image quality substantially. Using this technique,

the sample volume was determined to be a cuboidal region of interest that encompassed the entire monkey brain: Manual shimming was then performed using linear, second and higher-order polynomials. The results of such a shim are shown in Figure 7, where EPI slices and analogous anatomical images are comparable in global brain shape and local features. Each monkey subject has a unique shim parameter, and these provide a good initial shimming basis for each fMRI session.

The surface coil plays an important role in optimizing SNR. Features to be considered in designing or purchasing a coil include its size, position and shape. In choosing the *size* of the coil, a compromise must be made between greater SNR with smaller coils or greater depth of coverage with larger coils. Irrespective of size, the SNR will decrease with increasing distance from the center of the coil, thereby limiting the sensitivity of the coil to roughly its radius. Our custom-made coils are shown in Figure 8. The coils in Figure 8A and 8C are similar in size, and when positioned 1.0 cm above the ear bars, provide excellent full brain coverage. The oval coil in 8C is smaller in length and width than the circular coil (8A) and fits snugly around the circumference of the SM head, maximizing SNR for our preparation. The images shown in Figure 7, and fMRI data reported here, were taken with the coil shown in Figure 8C. The small coil in Figure 8B provides increased SNR over a small circumference and depth, and is used in applications requiring high resolution over small spatial volumes. All of the coils shown in Figure 8 are ‘receive-transmit’—they transmit RF pulses and receive the subsequent signal. Adjustable capacitors were incorporated into those shown in Figure 8B and 8C, to compensate for different loads. The coil in Figure 8A was tuned outside of the magnet to 400 MHz (Larmor frequency for the 9.4T magnet).

Anatomical MRI Paradigm

Anatomical imaging is most frequently performed with RARE (Rapid Acquisition Relaxation Enhancement) and MSME (multi-slice multi-echo) sequences. Using either of these SE sequences, high resolution and high gray-white matter contrast images are acquired. For our purposes, the RARE sequence is ideal for fast 2-D anatomy to align with fMRI EPI data. Examples of RARE images are shown in Figures 7 and 10A (bottom). The data shown in Figure 7 was acquired in 340 seconds and reveals the laminar structure of primary somatosensory cortex (SI: slice 5, arrow). The MSME sequence is a conventional SE anatomical acquisition, and requires a longer time scale (TR x 256, if a 256 x 256 matrix). Examples of MSME are shown in Figure 10A (top) and 10B, and also are used in later figures for overlaying fMRI statistical maps. Either sequence is used to collect data over the entire brain or a region of interest using slice thicknesses ranging from 80 - 500 μm .

Functional MRI Paradigm and Data Analysis

In our current studies, we have had success applying vibrotactile stimuli in a blocked design with alternate periods of stimulation (8 seconds) and no stimulation (12 seconds). The off-on pattern is repeated eight times (8 epochs) during a single functional scan for a total run length of 160 s. Due to the known decreases in BOLD signal with isoflurane anesthesia (Disbrow et al., 2000), averages across several runs are required to detect stimulus-evoked activity. All runs deemed acceptable (motion of less than 200 μm) are averaged for each stimulus condition (~ 10 - 15 runs of 160 s each) to create a grand average for each stimulus condition. Using an orthogonalized boxcar correlation and the *AFNI* software, the grand averaged time series is correlated with the hypothesized hemodynamic response function. The resulting statistical maps are typically smoothed at 625 μm (one pixel, in-plane) or not spatially smoothed.

Stimulus conditions include vibration of one focus on the distal digit tip, and of two foci placed on the distal and middle segment of the same digit, vibrated simultaneously or with inter-stimulus onset asynchrony of 100 ms offset (Nelson et al., 2005). The latter stimulus condition elicits the percept of tactile apparent motion in humans (Kirman, 1974; Aparicio and Moore, 2005).

A consistent challenge in functional imaging is to find an appropriate ‘significance’ level for the determination of functional activation. While the risk of false positives is high due to the enormous sample space (often thousands of voxels), many corrections are overly conservative (Locascio et al., 1997). To determine criteria for deeming activation statistically significant, we constructed statistical maps during ‘no stimulus’ presentation, in which a PZ embedded in an identical holder just distal to the hand was activated, but without direct skin contact. This stimulus condition is used to estimate the non-physiological noise potentially induced by the PZ elements. The runs were acquired in the same session in alternation with vibrotactile stimulation, and an equivalent number of ‘no’ stimulus runs were acquired (10 – 15). Using data from several sessions across 2 monkeys, we empirically defined the probability of aberrant activation in our scanning conditions using the coil shown in Figure 8C. Specifically, the correlation threshold level at which $p=0.005$ in the ‘no’ stimulation data—5 aberrant voxels are activated in 1000 voxels—is typically set as our threshold in the vibrotactile scans. Figure 9 shows an example of non-overlapping distributions of correlation values for a stimulus run versus a ‘no’ stimulus run. As noted by the black line and asterisk in this example, significance was determined at $r = .12$.

RESULTS

Anatomical images at 9.4T

The ability to obtain detailed anatomy is a clear advantage of imaging at high-field strengths. A hallmark of high-resolution brain MRI is the ability to detect layer IV in primary visual cortex. This signature feature is known as the stria of Genari (Gross, 1998), and reflects the dense cell body and thalamocortical axonal termination layer in primary visual cortex. The stria can be seen with the naked eye in unstained tissue, and is easily observed in images at high-field strengths. Compared with the prominent layer IV in visual cortex, layer IV in primary somatosensory cortex (SI) is more subtle. However, as shown in the coronal slices in Figure 10A (bottom), at 9.4T with an in-plane resolution of 195 μm , layer IV in SI is clearly defined. These coronal slices were taken at a slice traversing the central sulcus (CS: shown in a 3-D rendered brain in the top image in 10A). A clear macro-anatomical marker for the ‘Rolandic’ cortex of the SM can also be appreciated in this coronal image, the thickening and bending of the gray matter at approximately mid-way through its medio-lateral course.

Another preparation that we have found useful for high-resolution imaging is the post-mortem SM brain. While there are obvious issues in making inferences from this non-living brain tissue preparation, high-resolution anatomical scans can be run for several hours. An example of such an image is shown in Figure 10B (40 hour scan). In the post-mortem SM brain, layer IV in SI is readily observed (thin arrow), as are electrode track penetrations (thick arrow). This approach allows relatively precise localization of the track orientation and depth. Using high-field

imaging, electrode track information could also be obtained from living primates and could ultimately reduce the need for euthanasia and increase the lifespan of research monkey subjects.

Functional MRI at 9.4T

Electrophysiological maps in SM reveal discrete representations of the distal fingertips in areas 3b and 1, separated by ~2 - 3 mm in the anterior-posterior axis (Sur et al., 1982). Vibrotactile stimulation to the second digit tip was, therefore, predicted to evoke BOLD signal increases in areas 3b and 1, paralleling these maps. In the example shown in Figure 11, activation occurred in an anterior focus, putative area 3b, and at a region located ~2 mm posterior, putative area 1. Anatomical and electrophysiological considerations from other species suggest that the more posterior activation may also encompass part of area 2 (Pons et al., 1985). The distance between the fMRI activation foci is similar to the area 3b to 1 distance obtained from electrophysiology maps (Figure 11A, top). The ability to distinguish between these regions in the SM requires higher spatial resolution than is typically employed in human imaging at lower field strengths (e.g., 3 mm voxels: Moore et al., 2000; Nelson et al., 2004). In the medio-lateral direction, the location of each BOLD focus shows good correspondence to the location of the 2nd digit receptive field maps in areas 3b and 1, and the thickening of the central sulcus in Figure 11B (bottom) again provides an anatomical correlate of the Rolandic region.

Time courses for the putative area 3b and 1/2 activation clusters are shown in Figure 12. The figure on the left reveals the full time course over the eight stimulation periods (shaded in gray) for 3b and 1/2. The response patterns are similar for the two different clusters, including within-epoch parallels (e.g., see the 4th stimulation epoch). A decline of stimulus-evoked response towards the end of the EPI run was also observed, an effect commonly observed in human fMRI (CIM and AJN, unpublished observations). The ‘on-off’ cycle averaged across all 8 epochs is shown on the right and reveals a similar response in both regions.

The lateral sulcus of New World monkeys has several distinct somatosensory regions (Krubitzer et al., 1995). An example of SM activation in this region is shown in Figure 13 with recent data from the New World Titi monkey (top right: Coq et al., 2004). From the fMRI statistical map, three distinct activation foci were observed. In accordance with the electrophysiology map, two loci have been labeled the putative ventral somatosensory (pVS) and secondary somatosensory (pS2) regions. A third activation focus, located on the inferior bank of the lateral sulcus is labeled the putative caudo-medial region (pCM). This area may be the homologue of macaque area CM, a region that is responsive to both auditory and tactile stimulation (Schroeder et al., 2001).

While preliminary, our data also suggest that higher field imaging allows observation of activation at deeper anesthetic levels than at lower fields. Previous studies of lateral sulcus somatosensory regions in the human and macaque at 1.5T reported that 0.8 % isoflurane anesthesia suppressed all significant BOLD activation, even when using a GRE sequence (Disbrow et al., 2000). We have, however, consistently observed activation at higher isoflurane levels using SE imaging and the paradigms described above. The percent signal change at 0.65% and 1% were approximately equivalent, though the lower anesthetic level appeared to have a faster onset time.

CONCLUSION

Squirrel monkey imaging at 9.4T is a promising technique for non-invasive studies of the primate brain, and the anatomical and functional resolution obtained with this approach is complimentary to electrophysiological and optical techniques. We emphasize in closing that the SM model presented is applicable to studies beyond the tactile-related examples described. This model is also ideal for performing longitudinal studies in lesioned or pathological states. Future directions in our research include parallel sensory mapping in other modalities—we are currently conducting visual fMRI studies with a projection beam focused within the bore, and have obtained preliminary data demonstrating significant activation of multiple visual cortical areas. Another advantage of using a smaller animal in a higher field is the relatively greater resolution obtained in subcortical structures, making this preparation potentially ideal for studies of subcortical anatomy and functional organization of structures such as the thalamus and basal ganglia.

Another important future direction is the enlistment of contrast agents. While there is a clear advantage of using BOLD—because an identical measure can be obtained in humans—animal models permit the use of contrast agents that enhance functional signals and, potentially, provide a closer match to the electrophysiological signals of interest. To this end, we are beginning to scan with Dextran-coated Monocrystalline iron oxide nanoparticle (MION). This contrast agent has been used in repeat monkey and rat imaging studies where enhanced contrast to noise has been observed (Leite et al., 2002; Vanduffel et al., 2001), even at the high field strengths employed here (Mandeville et al., 2004).

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FIGURE LEGENDS

Figure 1. Animal positioning in cradle The scanning cradle is shown with a cartoon image of the basic monkey position. The arms are extended forward, and one hand is secured to the tactile stimulator hand mold, while the other is used for pulse oximetry measures. The cradle with foam rubber application on the base fits precisely in the diameter of the 11.7 cm 9.4 T bore. Arrows at each end of the cradle indicate the direction that specified cables exit the cradle.

Figure 2. Head motion Head motion plots (mm of displacement in the pitch direction) are shown for two functional scans. A clear reduction in head motion was observed following the use of the chin rest and head restraint elements (see Figure 8C). Motion of the amplitude observed in the ‘ear bar, coil’ condition, where ear bars and mild padding between the fixed coil and the head were employed, leads to non-usable functional data.

Figure 3. Vibrotactile stimulator A side profile schematic of the tactile stimulator in contact with the digit tip. The PZ element is mounted in a plastic brace, and a small plastic post that contacts the skin is slotted into a base affixed to the PZ element.

Figure 4. Stimulus calibrator *Left* A picture of the calibrator hardware set-up. Waveform signals are sent from the calibration software (portable computer, BSI) specifying the desired frequency and amplitude of PZ vibration. Actual PZ excursion is measured via an optical sensor mounted on a micromanipulator and captured by an analogue input PCI card (IOTech). An immovable steel platform is used to eliminate vibration that arises from non-PZ sources. *Right* A plot of PZ displacement as a function of driving voltage. These data were obtained while the monkey’s finger was in contact with the vibrating probe. The asterisk indicates the voltage (150 V) and displacement ($\sim 80 \mu\text{m}$) typically used in our tactile studies.

Figure 5. Spin echo versus gradient echo imaging Coronal images taken at identical slice positions using GRE (TR 2.0 s, TE = 13.0 ms) and SE (TR 2.0 s, TE = 25.4 ms) sequences. Signal loss at regions with high magnetic susceptibility is observed in the GRE images (arrow). FOV = 5.0 cm, 80 x 80 matrix, 625 μm , 1 mm slice thickness.

Figure 6. Single shot spin-echo pulse sequence The pulse sequence schematic displays the frequency encoding performed along the x axis (‘Read’) with 80 points and 100 μs rise time, the phase encode along the y axis (‘Phase’) with 80 phase encode lines, and the slice excitation (‘Slice’) surrounded by crusher gradients. The bottom line displays the occurrence of the first radiofrequency pulse (flip angle = 90^0) and the second refocusing pulse (flip angle = 180^0) that creates the spin echo with maximum amplitude at the echo time (TE). Data were sampled at TE = 25.4 ms. Typical EPI parameters used for fMRI data include a TR of 2.0 s; 17 coronal slices, 1mm thick; FOV is 5.0 cm; 80 x 80 acquisition matrix; reconstructed using 128 x 128 matrix with zero filling.

Figure 7. EPI and corresponding anatomical images The fMRI and corresponding anatomical images taken during a single imaging session with coil C in Figure 8. Numbers indicate slices moving from the anterior to posterior direction. Anatomical images were collected with a RARE sequence. To achieve the grey-white matter contrast shown in RARE images, the following parameters were used; TE=12.447 ms, TR=10000 ms, RARE factor=8, 256 x 256

matrix, 17 x 1 mm thick slices, 195 μ m in-plane, 1 mm thick; acquired in 340 s. The EPI images do not exhibit gross distortions in geometry and mirror the anatomical data in the right to left and superior to inferior dimensions. The arrow (slice 5) points to layer IV in primary somatosensory cortex.

Figure 8. Receive-transmit custom-made surface coils A. Top view of 7 cm circular coil mounted in plastic and secured to the cradle. This coil is used for 3-D anatomical imaging with a 6.4 cm FOV. B. A 2 x 1 cm oval coil. This coil is used for high-resolution anatomy and fMRI over a small region (FOV = 3.0 cm). The matching and tuning capacitors for coils A and B are accessed remotely by a tuning rod. C. A 6 x 5 cm oval coil (FOV = 5.0 cm). Also shown are the chin rest and Y-piece head restraint. Once a coil is positioned, the head piece is swiveled forward and secured by a second screw. The rubber end of the Y-piece sits at the level of the brow on the forehead and is manipulated in the anterior-posterior direction by a screw.

Figure 9. Correlation threshold criteria Plotted are the frequency distributions of correlation values for a vibrotactile condition and a 'no' stimulus condition, during which a PZ element attached to the hand holder was driven using the on/off paradigm but was not in direct contact with the skin surface. Thresholds in functional imaging studies were empirically determined using 'no' stimulus false positive distributions to define the correlation threshold cutoff for $p < 0.005$. In this example (one slice with ~ 1000 voxels), only responses in the stimulus condition with correlation values greater than $r = .12$ would be considered significant (black bar and asterisk).

Figure 10. Anatomical imaging, primary somatosensory cortex *Top* A three dimensional volume rendered image of a SM brain to display locations of the central sulcus (CS), lateral sulcus (LS) and superior temporal sulcus (STS). The 3-D image was acquired with coil A in Figure 8 using an MSME sequence. *Bottom* Coronal images taken through the central sulcus. Data were acquired using a RARE sequence, with an oval coil (3 x 2 cm, not shown) positioned unilaterally over the central sulcus (FOV 5.0 cm isotropic, 195 μ m in-plane, 1 mm slice thickness). Layer IV appears darker than the surrounding gray matter in these T2 images, indicating a higher density of white matter. B. An image from a post-mortem monkey brain imaged with small oval coil (coil B, Figure 8) taken through the central sulcus region. Note the prominent layer IV and the microelectrode track penetrating through all layers and white matter (arrows). Data were acquired using MSME (FOV 3.0 x 2.5 x 2.5 cm; matrix 300 x 256 x 256; isotropic resolution 100 μ m). Four echoes were collected (15 ms, 30 ms, 45 ms, 60 ms), data shown are from TE = 15 ms. The scan lasted 40 hours.

Figure 11. Functional imaging, primary somatosensory cortex A. *Top diagrams* Schematic figures from Sur et al., (1982) depicting areas 3b and 1 in the SM. *Top inset* A sagittal slice through a 3-D rendering showing the position of the central sulcus (yellow arrow). *Below* Functional activity overlaid on sagittal images showing activation of putative areas 3b and 1/2 (p3b and p1/2). B. p3b and p1/2 activation superimposed on coronal images. A characteristic thickening of the cortical mantle is localized to the p3b activation (see also Figure 10). The distance between the p3b and p1/2 activation regions is between 2 and 3 mm, as predicted by electrophysiology maps (A, top).

Figure 12. BOLD signal time courses for activation in putative areas 3b and 1/2 *Left* Time series from p3b and p1/2 averaged over 10 EPI runs. *Right* The average ‘on/off’ stimulus cycle (averaged from the full time series on the left). The vibrotactile stimulus ‘on’ period is indicated with gray background.

Figure 13. Functional imaging, lateral sulcus A. *Top left* An fMRI statistical map of three distinct activation foci in the lateral sulcus, the putative ventral somatosensory (pVS), second somatosensory (pS2), caudo-medial (pCM) areas. *Top right* A schematic from Coq et al., (2004) showing the position of tactile receptive fields and cortical areas in the lateral sulcus of New World Titi monkeys. *Bottom left* Average BOLD time courses for pVS voxels during tactile stimulation and also ‘no’ stimulation. B. An average BOLD time course for two sessions with different levels of isoflurane anesthesia. Session A (1.0% isoflurane) reveals a slower onset and an initial negativity in response to the vibrotactile stimulus than Session B (0.65%), differences that may reflect anesthetic concentration differences. The vibrotactile stimulus ‘on’ period is indicated with gray background.

Table I. Physiological measures (5 scan sessions, 3 - 6 hours each).

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