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Development of an apparatus and methodology for conducting functional magnetic resonance imaging (fMRI) with pharmacological stimuli in conscious rhesus monkeys

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Abstract

Functional magnetic resonance imaging (fMRI) is a technique with significant potential to advance our understanding of multiple brain systems. However, when human subjects undergo fMRI studies they are typically conscious whereas pre-clinical fMRI studies typically utilize anesthesia, which complicates comparisons across studies. Therefore, we have developed an apparatus suitable for imaging conscious rhesus monkeys. In order to minimize subject stress and spatial motion, each subject was acclimated to the necessary procedures over several months. The effectiveness of this process was then evaluated, in fully trained subjects, by quantifying objective physiological measures. These physiological metrics were stable both within and across sessions and did not differ from when these same subjects were immobilized using standard primate handling procedures. Subject motion and blood oxygenation level dependent (BOLD) fMRI measurements were then evaluated by scanning subjects under three different conditions: the absence of stimulation, presentation of a visual stimulus, or administration of intravenous (i.v.) cocaine (0.3 mg/kg). Spatial motion differed neither by condition nor along the three principal axes. In addition, maximum translational and rotational motion never exceeded one half of the voxel size (0.75 mm) or 1.5 degrees, respectively. Furthermore, the localization of changes in blood oxygenation closely matched those reported in previous studies using similar stimuli. These findings document the feasibility of fMRI data collection in conscious rhesus monkeys using these procedures and allow for the further study of the neural effects of psychoactive drugs.

Keywords

BOLD; fMRI; rhesus; conscious; cocaine; cortisol; prefrontal cortex; anterior cingulate

Introduction

Functional magnetic resonance imaging (fMRI) is a powerful translational technique that can be used to study biological processes in both human and non-human subjects (Bammer et al., 2005; Sava and Yurgelun-Todd, 2008). However, the neurophysiological basis of the signals measured by fMRI are poorly understood (Logothetis, 2002, 2003). A thorough

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understanding of these processes is most likely to be garnered from animal models wherein fMRI measurements can be concurrently obtained with direct measurements of electrophysiology and/or neurochemistry. Currently, invasive techniques that necessitate animal models are the only available means of obtaining these additional measurements (A.J. Schwarz, 2004). Therefore, despite the possibility of conducting fMRI experiments in human subjects, animal models will continue to be an integral component of the advancement of fMRI research. In addition to this consideration, animal models allow for a level of experimental control that is not always possible with human subjects. In particular, animal models facilitate the use of repeated measures designs that allow the researcher to study a process over time in the same subject. Finally, there is increasing interest in the use of neuroimaging to study pharmacological effects and the development of novel pharmacotherapies (Howell and Murnane, 2008; Howell and Wilcox, 2002; Tracey, 2001; Wise and Tracey, 2006). Much of this work will require the use of animal models.

There are at least four compelling reasons to carry out fMRI research in animals that are not anesthetized. The first is that this facilitates comparisons to human subject based fMRI experiments that are typically conducted in fully conscious subjects. Second, previous work has shown that anesthesia suppresses the signals measured with fMRI (Brevard et al., 2003). Third, the effects of anesthetics are likely to interact in profound ways with the pharmacological effects of other drugs deemed worthy of study. Finally, many important empirical questions require the subjects to actively engage in behavioral tasks. Therefore, the aim of the present work was to develop an effective apparatus and methodology that would allow fMRI studies to be carried out in fully conscious rhesus macaques.

The present study is not the first attempt to develop fMRI techniques applicable to conscious monkeys. However, the approach taken is distinct as these previous studies typically utilized techniques such as the surgical implantation of head posts or pins to minimize motion, the use of reversible anesthetics to initiate immobilization of the subject, or the use of injected contrast agents to increase the contrast to noise of the functional signal. (Andersen et al., 2002; Dubowitz et al., 1998; Ferris et al., 2001; Gamlin et al., 2006; Keliris et al., 2007; Pinski et al., 2005; Stefanacci et al., 1998; Vanduffel et al., 2001). While good quality fMRI data can be collected utilizing these procedures, the goal of the present work was to determine if good quality BOLD fMRI data could be collected using a distinct approach. This was deemed worthy of study because head posting requires costly and difficult surgical procedures, can lead to increased susceptibility artifacts, requires maintenance of surgical preparations, and may lead to medical complications that impair the health of the subject. Furthermore, head posting may be most effective when short duration stimuli, such as visual stimuli, are studied as this technique is enhanced by behavioral controls that train the subject to remain still during the stimulus presentation period (Keliris et al., 2007; Pinski et al., 2005). However, there is increasing interest in the use of fMRI to study pharmacology which necessitates the development of procedures suitable for the sustained timecourse of a pharmacological stimulus (Howell and Murnane, 2008; Wise and Tracey, 2006). Therefore, we sought to develop an apparatus that did not require head posting or initial pharmacological immobilization and thus could be used to study a sustained stimulus with a certainty that residual pharmacological effects were absent. The present work describes the neurobiological effects of the highly abused psychomotor stimulant cocaine, under these conditions.

While compelling reasons exist to acquire fMRI data in conscious subjects, there are also challenges inherent to these types of studies. Either subject stress or spatial motion can impair the quality of fMRI data. Within this study, extensive efforts were made to minimize any stress to the subjects. The results of these attempts are described as indexed through objective physiological metrics. In addition, subject motion poses a difficult challenge in

fMRI research utilizing conscious subjects; particularly when motion is correlated with the presentation of the stimuli (Andersen et al., 2002). The present work describes the results of the pre and post-acquisition techniques utilized to minimize the effects of subject motion. Particular attention was paid to developing an apparatus that did not require surgical implants or anesthetic immobilization. Subject stress due to these procedures was evaluated using objective metrics. Furthermore, subject motion and blood oxygenation level dependent (BOLD) fMRI measurements were evaluated by scanning subjects under three different conditions: the absence of stimulation, presentation of a visual stimulus, or administration of i.v. cocaine (0.3 mg/kg). This work documents the feasibility of carrying out fMRI studies in fully conscious rhesus monkey using these procedures.

Methods

Subjects

Three adult female rhesus monkeys (*Macaca mulatta*) served as subjects for these studies. All three subjects had a history of exposure to psychoactive compounds and engagement in behavioral experiments. The monkey colony was maintained at an ambient temperature of $22 \pm 2^\circ\text{C}$ at 45–50 % humidity, and lights were set to a 12 h light/dark cycle. Each subject was individually housed and free-fed Purina monkey chow (Ralston Purina, St. Louis, MO), supplemented with fresh fruit and vegetables and water was available *ad libitum* within the colony. Food intake was monitored daily throughout the study by recording the number of chow delivered during each afternoon feeding and the number of chow remaining in the home cage each morning. Any food not consumed by the subject was removed at midnight of the morning preceding an acclimation or data collection procedure. Subjects were closely monitored both during experimental procedures and within the colony for presentation of symptoms consistent with pain or distress. All studies were carried out in accordance with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health, and experimental protocols were approved by the Animal Care and Use Committee at Emory University.

Surgery

Each subject was implanted with a chronic indwelling venous catheter and vascular-access port into the femoral or jugular vein under sterile surgical conditions as previously described (Howell and Wilcox, 2001). Catheters were regularly flushed with heparinized saline (100 U/mL) to maintain patency.

Apparatus

In order to acquire quality imaging data without subject motion or stress, specific attention was paid to the development of an apparatus suitable for conducting fMRI studies in conscious rhesus monkeys. The design and implementation of the apparatus was a compromise between rigid head fixation and the maintenance of physiological stability. The frame of this custom apparatus was built out of cylindrical polyvinylchloride (height = 55.9 cm, inner diameter = 30.2 cm, and thickness = 0.6 cm; Figure 1, A) and was designed with a detachable front section, a track for a primate collar (length = 15.0 cm, width = 9.5 cm, and height = 1.0 cm) through the top plate (thickness = 3.8 cm) of the frame, and a detachable rear block that allowed it to securely attach to a standard primate chair (Primate Products, Woodside, CA). This design allows subjects to be moved using a standard “pole and collar” technique from their home cage into the restraint cradle without the use of anesthetic immobilization. Subjects could then be transported from the colony to the laboratory or imaging suite. During a procedure, the head was immobilized by a silicone rubber mold (Smooth-Sil 940; Smooth-On, Easton, PA) that was specifically formed for each subject. This mold was created by first forming a near-exact replica of each subject’s head out of

plaster as previously described (Howell et al., 2001). The rubber mold was formed to this replica by applying the rubber in a liquid state around the cast and applying a platinum catalyst to transition the rubber to solid state with a shore hardness of 40A. This hardness was previously determined to provide sufficient head fixation without imposing undue stress to the subject. Figure 1, B shows a side profile of an example plaster replica of the head for subjects RBp3. The replica occupies the same space that the head would occupy during a procedure. The replica is partially surrounded by one half of the mold and is positioned as if the subject were looking upwards in the image. Two slits were cut in the mold to allow the subject to ventilate and see visual stimuli. The slit on the right is above the portion of the replica that matches the position of the eyes. The mold was supported on all four sides by acrylic plates that have been formed to fit flush with the side of the mold (Figure 1, C). The dorsal (length = 1.1 cm, width = 6.7 cm, and height = 11.7 cm) and lateral side plates (length = 1.1 cm, width = 6.0 cm, and height = 12.4 cm) are flat plates that can be used to add functionality, such as fiducial markers, to the apparatus. Furthermore, these plates contain threaded fiberglass screws that extend through the entire top plate of the apparatus and were used to buttress its structural integrity via the attachment of oversized wing nuts. The ventral plate is curved and fits over the section of the mold that covers the jaw. This piece was used to grossly position the subject in the mold. The subject, surrounding mold, and accessory plates are covered by a (height = 15 cm, inner diameter = 13.3 cm, and thickness = 0.5 cm) polyvinylchloride cylinder designed to fit within the imaging coil (Figure 1, D). Body motion poses a particular challenge for fMRI experiments in conscious subjects as previous studies have shown that, even in the absence of head movements, body motion can disrupt the homogeneity of the magnetic field and produce both image and apparent motion artifacts (Gamlin et al., 2006; Keliris et al., 2007). Here, body motion was reduced by creating an Alpha Cradle IHI/CNR foam insert designed to fill the void in the imaging apparatus not occupied by the body (Smithers Medical Products, Canton, OH). The use of foam inserts has been shown to effectively reduce either head (Howell et al., 2001) or body motion (Gamlin et al., 2006). The insert was reinforced by soft padding and nylon straps that are placed over the torso of the subject. Importantly, the head was maintained closely shaved as this facilitates veterinary examination after each procedure and may serve to keep the subject cooler during the procedure. Furthermore, the scan room was kept cool during imaging sessions and additional cool air was blown over the torso through a series of ventilation tubes (Figure 1, E) in an effort to prevent the subject from overheating during the procedure. Subjects were placed within this cradle in the prone position on the bed of the MR scanner (Figure 1, F).

Animal habituation protocol

In order to minimize motion and stress, all subjects were extensively and gradually habituated to all procedures necessary for these experiments over a period of several months. Every effort was made to make these protocols routine procedures. The subjects had a previous history of engaging in behavioral experiments and therefore were acclimated to standard primate chairs and “pole and collar” procedures at the initiation of the study. Subjects were first acclimated to transportation within the frame of the custom apparatus, the research staff that carried out this study, and the laboratory that contained a mock fMRI chamber. Subjects were initially placed in the restraint apparatus and brought to the laboratory for 30 minute sessions three times per week. Over the next month, an increasing number of the pieces of the apparatus were added from session to session until the subject was finally placed in the entire setup for several sessions. Then, the subject was placed into a customized chamber designed to simulate many aspects of the actual scanner (i.e. mock fMRI chamber). Over the next month, the duration of the procedure was gradually increased from 30 minutes to 2 hours and the frequency of immobilization was reduced from 3 times per week to 1 time per week. Over the next several weeks, subjects were acclimated to the

noises produced by the MR scanner via audio playback of recordings of several MR pulse sequences (anatomical and functional) at sound levels that were gradually increased to approximate those produced by the MR scanner (up to 110 dB). Importantly, the surrounding head covering provided significant sound attenuation (10 to 20 dB) as this has been shown to be important for fMRI data quality (Andersen et al., 2002). The terminal phase of acclimation involved transportation to the Yerkes imaging center where the subject was immobilized and several sessions were undertaken to expose the subject to the scanner environment prior to the collection of experimental data. Once fully acclimated to the procedure, subjects were habituated to the procedures necessary to obtain physiological measurements. These measurements were subsequently collected over several sessions to objectively evaluate the stress to the subject (see Results). Finally, subjects were acclimated to administration of i.v. cocaine (0.3 mg/kg) within the apparatus at least 3 times prior to imaging.

Physiological measurements

In order to objectively evaluate whether subjects were experiencing heightened levels of stress, physiological measures were taken when the subjects were in the restraint apparatus and compared to those obtained when the subjects were restrained in a standard primate chair. In each subject, physiological measurements were taken three times over a restraint period of two hours in each condition and averaged (Figure 2, A - D). In all subjects, the three physiological measurements in the fMRI cradle were acquired first and then measurements were taken in the commercial chair. These physiological measurements were first acquired in the custom cradle and then the commercial chair in all subjects. Heart rate data were obtained by securing a pulse oximetry probe to the tail via Vetwrap (3M, St. Paul, MN). Systolic, diastolic, and mean arterial blood pressure data were collected via placement of a non-invasive blood pressure cuff on the right biceps muscle. This cuff was automatically inflated every five minutes during the session. Rectal temperature data were collected via placement of a sheathed and lubricated temperature probe 5 cm into the rectum. Respiratory data were collected via placement of a small bore line close to the nose of each subject. This line was secured by a custom built plastic arm that attached to the lateral side plates and held the line close to the nose of each subject. Since the small bore line must remain close to the nose of the subject in order to reliably measure respiration and subjects can freely move their head while in a standard primate chair, a potentially problematic system would have to be designed to modify this system for respiratory data collection in a standard primate chair. Therefore, respiratory data were compared in separate sessions when the subject was in the restraint apparatus with or without the custom head mold. All subjects were acclimated to these procedures over several sessions prior to the collection of experimental data. The heart rate and respiratory rate signals were collected by a SurgiVet V90041 (Smiths Medical, St. Paul, MN) physiological monitor and fed into a desktop PC running AcqKnowledge 3.7.3 (BIOPAC, Santa Barbara, CA) for real-time recording. Blood pressure and temperature data were collected by a SurgiVet V9200 (Smiths Medical, St. Paul, MN) physiological monitor and manually recorded by research personnel every five minutes. Potential stress was also objectively evaluated via measurement of plasma cortisol levels (Figure 2, E). All subjects were surgically fitted with chronic indwelling venous catheters (see surgery section). Plasma cortisol levels were determined twice in each subject. Baseline levels were taken at the beginning of each procedure by moving the subject to the respective apparatus and immediately transporting the subject to a veterinary procedure room adjacent to the colony. Blood was collected in less than ten minutes. Furthermore, the colony was not disturbed for at least 90 minutes prior to the session. After baseline cortisol level blood sample collection, subjects were immobilized for two hours and blood was collected every 30 minutes. Subjects were habituated to this procedure for several sessions prior to experimental data collection. Samples were assayed by the Yerkes National Primate Research Center's

Biomarkers Core Laboratory using a radioimmunoassay as previously described (Sanchez et al., 2005). All endocrine measurements were collected at approximately 10:00 am regardless of condition. The order of endocrine measurements was counterbalanced across condition.

Physiological data analysis

Heart rate, respiratory rate, mean arterial blood pressure, and rectal temperature were taken over three sessions whereas endocrine measurements were taken over two sessions. One-way repeated measures analysis of variance (RM ANOVA) was used to determine if there were significant differences from session to session. Data from each session were then averaged. Subsequently, a two-way RM ANOVA was then used to determine if there were significant differences as a function of the apparatus the subject was in or the time spent in a given apparatus. Post-hoc analysis was carried out via a one-way RM ANOVA with correction for multiple comparisons by the Tukey's test. Graphical presentation of all data depicts mean $\pm$ SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data. All graphical data presentations were created using GraphPad Prism 4 (La Jolla, CA), all statistical tests were performed using SigmaStat 3 (San Jose, CA), and significance was arbitrated at a $P < 0.05$. All data were binned into 30 minute segments for graphical presentation and data analysis.

fMRI data acquisition

Scans were conducted in a Siemens (Siemens Healthcare, Erlangen, Germany) Trio 3 Tesla magnet with 90cm bore using a (19 cm inner diameter) Siemens CP extremity coil. Anatomical images were acquired using a 3D single-shot magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence optimized for T1 contrast. Scan parameters were as follows: TR = 2700ms, TI = 800, 192X192 Matrix, 96mm FOV, 1 NEX, 190 Hz per pixel Bandwidth, 8 degree flip angle, and 9% frequency oversampling yielding a final isotropic resolution of 0.5mm. At least 10 separate collections were averaged off-line for each subject to generate the final anatomical image used for coregistration of the functional data. Anatomical images were acquired in awake subjects in the imaging apparatus to facilitate coregistration to the functional images. BOLD images were collected utilizing a gradient echo single-shot echo planar imaging (EPI) sequence; collected after a standard second order shim. These 2D T2*-weighted images were acquired with the following parameters: 47 slices, TR = 4 seconds, TE = 40ms, 64 $\times$ 64 data matrix, 96mm X 96mm FOV, slice thickness = 1.5mm with no slice gap, 1594 Hz per pixel Bandwidth, and 90 degree flip angle yielding a final isotropic resolution of 1.5mm. The first 2 measurements in each time series were discarded to ensure steady state measurements. Furthermore, a saturation pulse was applied during each acquisition to minimize extraneous effects at the boundary of the cranium. Finally, field inhomogeneities were mapped using a standard Siemens phase and magnitude image collection sequence for later correction of any EPI image distortions (Figure 3). These scan parameters provide a balance between signal, contrast, resolution, and facilitate co-registration to functional data. In particular, a single-shot EPI sequence was used to minimize the influence of subject motion. Furthermore, relatively small and isotropic voxels were used to minimize the effects of motion and to facilitate coregistration to anatomical images, respectively. Finally, a 40 ms TE was found to enhance the sensitivity of BOLD signal measurements at 3T without resulting in excessive signal drop-off.

Baseline motion

Each subject underwent three different fMRI acquisitions. To evaluate baseline motion, a scan was obtained in the absence of any stimulation. This scan was composed of 100 image acquisitions and lasted 6.67 minutes (100 acquisitions X 4 second TR = 400 seconds).

Visual stimulation

Subjects were presented with an alternating checkerboard visual stimulus designed to elicit activation of visual cortex (Logothetis, 1999). This stimulus was composed of alternating full contrast black and white squares. Stimuli were presented in four blocks composed of a 60 second epoch without stimulation, followed by a 30 second epoch of stimulus presentation, and terminating with an additional 60 second epoch without stimulation. The stimulus was alternated at 5 Hz and occupied 15 degrees of the visual field. Stimuli were presented using a Pentium III workstation under timing control by Presentation software (Neurobehavioral Systems, Albany, CA), at a resolution of 640 X 480 pixels, and at a frame rate of 60 Hz. Subjects were allowed to freely view the stimulus presentation and were not previously trained to fixate or otherwise attend to the stimulus.

Cocaine administration

Baseline data were collected for two minutes, followed by a saline infusion, followed by 2 minutes of additional scanning, followed by an intravenous infusion of cocaine HCl (0.3 mg/kg), and 6 minutes of subsequent scanning. This paradigm allowed for a within session negative control (i.e. saline infusion) and was based on previous data showing a robust increase in blood flow within 5 minutes of a cocaine bolus (Howell et al., 2001; Howell et al., 2002; Howell et al., 2009). Cocaine HCL was supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC) and dissolved in 0.9% saline. Throughout this study the infusion rate and volume were held constant at 15 ml/min and 4ml respectively. This dose is expressed as the salt form.

Spatial motion analysis

Translation and rotation data were determined during each of the three scans and analyzed separately. The maximum translation and rotation from one acquisition to the next across the entire time series and across all three scans was compared to specific criteria (translations to one half the size of the voxel size or 0.75 mm and rotations to 1.5 degrees) via a one sample t-test. Furthermore, two-way RM ANOVA was utilized to compare the maximum, mean, and the variability of translational and rotational motion across axis and scan condition. For these analyses realignment parameters were transformed by taking the absolute value of the difference from one acquisition to the next and therefore represent absolute motion across acquisitions. Graphical presentation of all data depicts mean $\pm$ SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data. All graphical data presentations were created using GraphPad Prism 4 (La Jolla, CA), all statistical tests were performed using SigmaStat 3 (San Jose, CA), and significance was arbitrated at a $P < 0.05$.

fMRI data analysis

Analyses were carried out using the standard image analysis package Statistical Parametric Mapping version 5 (SPM5 – Wellcome Trust Center for Neuroimaging, London, UK) supplemented by custom software written in the matrix based programming environments IDL (ITT, Boulder, CO) and MATLAB (MathWorks, Natick, MA). Preprocessing of the images was initiated via placement of both the anatomical and functional images in AC-PC alignment and in gross registration to one another. Time series realignment using a 6 parameter rigid body algorithm (Cox and Jesmanowicz, 1999; Woods et al., 1993) to reduce the influence of any subject motion was then carried out. Concurrently, field inhomogeneity data were used to correct any geometric distortions in the EPI images using an automated algorithm that takes into account the interaction between motion and inhomogeneities and has been shown to result in an improved coregistration between EPI and T1 images (Cox and Jesmanowicz, 1999; Hutton et al., 2002). Anatomical data were

then segmented into gray matter, white matter, and bias corrected images. Functional data were then spatially normalized to the bias corrected (intensity normalized) anatomical images and spatially smoothed using a kernel with a full width at half max equal to two times the native resolution of the image (i.e. 3mm). Linear drift was accounted for by global normalization across the time series and high-pass filtering. Whole brain analysis was carried out on a pixel by pixel basis using a parametric general linear statistical model. This analysis was confined to gray matter pixels using a custom generated mask to exclude any white matter or ventricle pixels that was applied to the data prior to statistical analysis. Motion parameters were used as covariates within this model to remove the influence of subject motion on the subsequent results. The general linear model fit was based on a flexible boxcar design using the canonical hemodynamic response function and corrections for multiple comparisons were carried out such the probability of a type I error was maintained at 5% (Genovese et al., 2002). Finally, the timecourse of the MR signal was determined in the voxel that showed the local maximum correlation to presentation of the visual stimulus (in visual cortex) or administration of cocaine (in the anterior cingulate). The signal measured under each condition was averaged across all three subjects. Graphical data presentations were created using GraphPad Prism 4 (La Jolla, CA).

Results

Under the conditions employed, rhesus monkeys could be reliably acclimated to undergo fMRI scans while awake. The integrity of the imaging data necessitated that subjects were minimally stressed and near motionless. To objectively assess the effectiveness of the training procedure in minimizing any stress to the subject, physiological and endocrine measurements were taken, in fully acclimated subjects, over two hour sessions in either the custom fMRI apparatus or in a standard primate chair (with the exception of the respiratory rate data – see Methods). In each condition, physiological measurements were taken over three sessions whereas endocrine measurements were taken over two sessions. In the custom fMRI cradle, one-way RM ANOVA reveal no main effect of heart rate ($F_{2,2} = 0.295$; $p = 0.760$), respiratory rate ($F_{2,2} = 2.027$; $p = 0.212$), blood pressure ($F_{2,2} = 0.051$; $p = 0.951$), and temperature ($F_{2,2} = 5.528$; $p = 0.096$) as a function of session. The powers of these tests were 0.051, 0.155, 0.051, and 0.214, respectively. In the primate chair (or custom apparatus without head restraint for respiratory rate data), heart rate ($F_{2,2} = 2.537$; $p = 0.194$), respiratory rate ($F_{2,2} = 2.501$; $p = 0.125$), blood pressure ($F_{2,2} = 2.154$; $p = 0.213$), and temperature ($F_{2,2} = 0.967$; $p = 0.454$) were not significantly different as a function of session. The powers of these tests were 0.177, 0.586, 0.158, and 0.051, respectively. Data from each session were then averaged. A two-way RM ANOVA was then used to determine if there were significant differences as a function of the apparatus used or the time spent in a given apparatus. Heart rate ($F_{2,1} = 0.074$; $p = 0.811$), respiratory rate ($F_{2,1} = 0.342$; $p = 0.618$), blood pressure ($F_{2,1} = 1.875$; $p = 0.304$), rectal temperature ($F_{2,1} = 0.002$; $p = 0.968$), and plasma cortisol levels ($F_{2,1} = 1.854$; $p = 0.306$) did not significantly differ by condition. The powers of these tests were 0.058, 0.085, 0.096, 0.058, and 0.095, respectively. Furthermore, there was no main effect of time spent in the apparatus for heart rate ($F_{2,3} = 0.395$; $p = 0.762$), respiratory rate ($F_{2,3} = 3.156$; $p = 0.107$), blood pressure ($F_{2,3} = 1.152$; $p = 0.402$), rectal temperature ($F_{2,3} = 0.402$; $p = 0.757$), or plasma cortisol levels ($F_{2,4} = 2.230$; $p = 0.155$). The powers of these tests were 0.050, 0.636, 0.066, 0.051, and 0.234, respectively. Mean basal plasma cortisol levels were, prior to research personnel entering the colony, were 22.925 ± 2.764 and 26.313 ± 4.202 micrograms per deciliter on the days when measurements were subsequently collected in the custom cradle or the commercial chair, respectively. Analysis via a paired t-test revealed that basal plasma cortisol levels did not differ across these different days ($t_3 = -3.388$; $p = 0.148$). The power of this test was 0.230.

In addition to a stable physiology, good quality fMRI data requires minimal subject motion. Figure 4 shows transformed realignment parameters across the three translational and rotational axes, assuming rigid body motion, averaged across the three subjects. These data are summarized in Table 1 as expressed by the maximum, mean, and standard deviation of the motion from acquisition to acquisition in each axis. One-sample t-tests revealed that translational and rotational movements were significantly less ($p < 0.05$) than criterion for all axes and conditions except Z-axis translations ($t_3 = -3.066$; $p = 0.092$) and X-axis rotations ($t_3 = -1.537$; $p = 0.264$) during visual stimulation. Two-way RM ANOVA revealed that, for the maximum translational motion from scan to scan, there was no main effect of axis (X, Y, Z; $F_{2,2} = 2.500$; $p = 0.197$) or condition (No stimulation, visual stimulation, cocaine; $F_{2,2} = 2.257$; $p = 0.221$) and no significant interaction ($F_{2,4} = 0.901$; $p = 0.507$). The powers of these tests were 0.174, 0.153, and 0.050, respectively. Furthermore, there was no main effect of axis ($F_{2,2} = 0.156$; $p = 0.860$) or condition ($F_{2,2} = 1.894$; $p = 0.264$) and no significant interaction ($F_{2,4} = 0.476$; $p = 0.753$) for maximum rotations. The powers of these tests were 0.051, 0.123, and 0.050, respectively. Mean translational motion from acquisition to acquisition showed a main effect of axis ($F_{2,2} = 7.623$; $p = 0.043$) but not condition ($F_{2,2} = 2.462$; $p = 0.201$) and there was no significant interaction between these factors ($F_{2,4} = 1.473$; $p = 0.297$). The powers of these tests were 0.580, 0.171, and 0.113, respectively. However, post hoc analysis via the Tukey's test did not show any significant individual differences ($p < 0.050$) between the three axes. Moreover, mean rotational motion showed no main effect of axis ($F_{2,2} = 1.220$; $p = 0.386$), condition ($F_{2,2} = 0.582$; $p = 0.600$), and no significant interaction ($F_{2,4} = 1.936$; $p = 0.198$). The powers of these tests were 0.068, 0.051, and 0.185, respectively. The variability (standard deviation) of translational motion also did not vary as a function of axis ($F_{2,2} = 4.762$; $p = 0.087$) or condition ($F_{2,2} = 3.051$; $p = 0.157$) and there was no significant interactions between these factors ($F_{2,4} = 1.233$; $p = 0.370$). The powers of these tests were 0.369, 0.222, and 0.079, respectively. Finally, the variability of rotational motion did not show a main effect of axis ($F_{2,2} = 0.900$; $p = 0.476$), condition ($F_{2,2} = 2.189$; $p = 0.228$), and no significant interaction ($F_{2,4} = 0.654$; $p = 0.641$). The powers of these tests were 0.051, 0.148, and 0.052, respectively.

Functional activity was assessed via BOLD fMRI signal changes following visual stimulation or acute cocaine challenge in all three subjects. Presentation of the visual stimulus elicited activation that was principally localized to visual cortex (Figure 5). These results remained after correction for multiple comparisons and were consistent across the three subjects. Furthermore, cocaine challenge elicited activation that was principally localized to the anterior cingulate and the dorsal regions of the prefrontal cortex (Figure 6). While there was some individual variability in the response to cocaine, these results also survived correction for multiple comparisons and were consistent across the three subjects. Furthermore, cocaine administration sporadically activated temporal and parietal regions of the cerebrum. Finally, the timecourse of the response to either visual stimulation (in visual cortex) or administration of cocaine (in the anterior cingulate) showed that the signal was stable and followed the known temporal dynamics of the BOLD response.

Discussion

The present study documents the utility of the described apparatus and procedures for the conduct of fMRI experiments in conscious nonhuman primates. An effective restraint device was developed that facilitated immobilization by readily attaching to a standard primate chair. Specific attention was paid to developing an apparatus that did not require the subjects to be surgically fitted with head posts. This reduced the invasiveness of the procedure, the need for a surgical preparation that may be associated with veterinary medical complications, and the possibility of surgical preparation related susceptibility artifacts. Furthermore, this device allowed for the immobilization of the subject without initial

anesthetic induction. Therefore, subjects could be scanned in the absence of residual anesthetic effects that may represent a significant confound, particularly in the context of pharmacological imaging.

High quality fMRI data requires stable physiology and minimal spatial motion. The effectiveness of the acclimation procedure in allowing subjects to meet these requirements was assessed via objective metrics. Direct comparisons were made for physiological parameters during immobilization in the restraint apparatus to immobilization in a standard primate chair. Heart rate, mean arterial blood pressure, rectal temperature, and plasma cortisol levels did not differ between these conditions. Furthermore, respiratory rate was unaffected by encasement of the entire head in an individually fitted mold. These data indicate that these subjects were not more stressed by these custom procedures than by standard primate immobilization procedures. Spatial motion was assessed by determining the absolute translational and rotational motion during the three different scan conditions. Imaging data was acquired in the absence of stimulation, during presentation of a visual stimulus, and both before and after intravenous administration of cocaine (0.3 mg/kg). Spatial motion did not significantly differ as a function of these conditions. Furthermore, motion did not significantly differ along the three spatial axes. Mean translation motion across the three conditions and the three axes varied from 0.009 to 0.07 mm whereas mean rotational motion varied from 0.07 to 0.20 degrees. Mean translation motion across the three conditions and the three axes varied from 0.05 to 0.33 mm whereas mean rotational motion varied from 0.39 to 0.91 degrees. Moreover, over the course of the three scans, the maximum translational and rotational motion, from acquisition to acquisition, never exceeded one half of the voxel size (0.75 mm) nor 1.5 degrees, respectively. These data indicate that the subjects remained relatively motionless during these procedures. These results clearly document that utilizing these procedures rhesus monkeys can be reliably acclimated to undergo fMRI experiments while awake and with minimal stress or motion.

High quality functional (EPI) and anatomical (T1W) images were also reliably acquired with these procedures. These images showed good signal to noise and relatively little artifact from motion or susceptibility differences. However, EPI image acquisition techniques are inherently sensitive to field inhomogeneities and thereby produce geometric distortions in the resultant image. Therefore, a field mapping technique was used to determine the vectors of the magnetic field inhomogeneities. The EPI images were then corrected by applying the inverse of the calculated field map. These procedures resulted in images that were a closer spatial approximation of the anatomy of an individual subject and have been shown to improve coregistration of the two image types (Hutton et al., 2002; Jezzard and Balaban, 1995). In addition, the anatomical specificity of stimulus induced neural activation was verified by presenting the subjects with a visual stimulus. Similar stimuli have been shown to principally increase blood oxygenation in visual cortex (Logothetis et al., 1999). The results of this study corroborate these findings by showing that the areas of significant blood oxygenation changes were largely localized to visual cortex. Taken together, these data show that under these procedures good quality images could be obtained and validate the anatomical specificity of the measured changes in blood oxygenation.

Administration of 0.3 mg/kg of cocaine resulted in a significant increase in blood oxygenation of the anterior cingulate and the dorsal regions of the prefrontal cortex. This effect is consistent with cocaine induced changes in cerebral blood flow in unanesthetized rhesus monkeys (Howell et al., 2002; Howell and Murnane, 2008; Howell et al., 2009). Furthermore, the cerebral metabolic changes engendered by cocaine in unanesthetized rhesus monkeys also show localization to the dorsal regions of the prefrontal cortex (Howell et al., 2009). The concordance of these data sets further supports the anatomical specificity of the changes in blood oxygenation reported in this study.

The brain activation pattern of cocaine measured in this study also shows considerable concordance with measurements taken in both human (Breiter et al., 1997; Kufahl et al., 2008; Kufahl et al., 2005) and rodent (A.J. Schwarz, 2004 ; Marota et al., 2000) subjects as these studies consistently show that mesocortical dopaminergic pathways are activated by cocaine administration. However, discrepancies also exist between these results. These differences may be due to the dose of cocaine administered, the time frame used for data analysis, the drug history of the subjects examined, species differences, the use of anesthetics, or other factors. The presently described methodology provides an excellent opportunity to study the influence of these factors on the neurobiological effects of cocaine. For example, the influence of drug history could be determined by training subjects to self-administer cocaine. Alternatively, studies could be carried out, using identical subjects and methods, to determine the influence of anesthesia on the BOLD response to cocaine – or non-pharmacological stimuli. Further research into these important issues is clearly warranted.

The brain activation pattern of cocaine may be different from the patterns produced by related psychomotor stimulant drugs of abuse such as amphetamine and methylenedioxymethamphetamine (MDMA). Specifically, amphetamine appears to produce a more widespread activation of regions innervated by dopaminergic terminals (Jenkins et al., 2004; Schwarz et al., 2007) whereas MDMA has a serotonergic component that may be lacking in the response to either amphetamine or cocaine (Brevard et al., 2006; Meyer et al., 2006). However, these studies were carried out with significant methodological differences. Nevertheless, if important differences exist in the brain activation patterns produced by different drugs of abuse it may have important implications for understanding the behavioral, subjective, and cognitive effects of these drugs and for the development of medications that attenuate the addictive properties of these substances. As such, further studies that determine if different drugs of abuse produce different brain activation patterns within subject and within methodology are warranted. Furthermore, if brain activation differences are verified, those results would call for the determination of the pharmacokinetic and pharmacodynamic mechanisms that mediate those differences. The results of the present work greatly enhance the feasibility of carrying out these studies.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Table 1

Translational (mm) and rotational (degrees) motion parameters collected under three different scan conditions: the absence of stimulations, presentation of a visual stimulus, or administration of an acute intravenous bolus of cocaine (0.3 mg/kg).

Variable	Axis	No stimulation	Visual stimulation	Cocaine
Maximum	X Translation	0.09 (0.02)	0.17 (0.003)	0.05 (0.03)
	Y Translation	0.20 (0.07)	0.24 (0.08)	0.07 (0.06)
	Z Translation	0.15 (0.06)	0.33 (0.14)	0.10 (0.09)
	X Rotation	0.44 (0.17)	0.91 (0.38)	0.68 (0.08)
	Y Rotation	0.39 (0.11)	0.73 (0.11)	0.70 (0.21)
	Z Rotation	0.54 (0.06)	0.54 (0.03)	0.71 (0.21)
Mean	X Translation	0.02 (0.005)	0.03 (0.005)	0.009 (0.007)
	Y Translation	0.05 (0.01)	0.06 (0.02)	0.01 (0.01)
	Z Translation	0.03 (0.01)	0.07 (0.03)	0.02 (0.01)
	X Rotation	0.12 (0.03)	0.18 (0.06)	0.14 (0.02)
	Y Rotation	0.07 (0.02)	0.13 (0.03)	0.08 (0.02)
	Z Rotation	0.16 (0.03)	0.17 (0.03)	0.20 (0.05)
Standard deviation	X Translation	0.02 (0.005)	0.03 (0.004)	0.008 (0.006)
	Y Translation	0.04 (0.01)	0.06 (0.02)	0.01 (0.01)
	Z Translation	0.03 (0.01)	0.07 (0.03)	0.02 (0.01)
	X Rotation	0.09 (0.03)	0.17 (0.06)	0.12 (0.02)
	Y Rotation	0.07 (0.02)	0.13 (0.02)	0.10 (0.03)
	Z Rotation	0.12 (0.02)	0.13 (0.02)	0.16 (0.05)