

cocaine bolus (Howell et al., 2001, 2002, 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 4 ml, respectively. This dose is expressed as the salt form.

2.6. 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°) 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 $p < 0.05$.

2.6.1. 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. 3 mm). 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).

3. 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 2 h sessions in either the custom fMRI apparatus or in a standard primate chair (with the exception of the respiratory rate data—see Section 2). 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 $\mu\text{g/dl}$ 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. Fig. 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 ($F_{2,2} = 0.156$;