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A method for conducting functional MRI studies in alert nonhuman primates: initial results with opioid agonists in male cynomolgus monkeys

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Abstract

Functional magnetic resonance imaging (fMRI) has emerged as a powerful technique for assessing neural effects of psychoactive drugs and other stimuli. Several experimental approaches have been developed to use fMRI in anesthetized and awake animal subjects, each of which has its advantages and complexities. We sought to assess whether one particular method to scan alert post-anesthetized animals can be used to assess fMRI effects of opioid agonists. To date, the use of fMRI as a method to compare pharmacological effects of opioid drugs has been limited. Such studies are important because mu and kappa opioid receptor agonists produce distinct profiles of behavioral effects related both to clinically desirable endpoints (e.g. analgesia) and to undesirable effects (e.g. abuse potential). This study sought to determine whether we could use our fMRI approach to compare acute effects of behaviorally equipotent (3.2 µg/kg) intravenous doses of fentanyl and U69,593 (doses that do not affect cardiorespiratory parameters). Scans were acquired in alert male cynomolgus macaques acclimated to undergo fMRI scans under restraint, absent excessive stress hormone increases. These opioid agonists activated bilateral striatal and nucleus accumbens regions of interest. At the dose tested, U69,593 induced greater left nucleus accumbens BOLD activation than fentanyl, while fentanyl activated left dorsal caudate nucleus more than U69,593. Our results suggest that our fMRI approach could be informative for comparing effects of opioid agonists.

Keywords

Macaque; fMRI; fentanyl; U69; 593; Awake animal imaging

Functional magnetic resonance imaging (fMRI) has emerged as a powerful technique for assessing neural effects of drugs and other stimuli. Several fMRI approaches have been developed to use in anesthetized and awake animal subjects, each of which has its

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advantages and complexities. Many prior macaque studies involving awake subjects have surgically affixed immobilization hardware to subjects' heads (e.g., Zhang et al., 2000; Ku et al., 2011; Chen et al., 2012), obviating the need for anesthesia or extensive training necessary to acclimate subjects to restraint procedures. Although this mode of restraint is quite successful in limiting head motion, it has not been clarified whether it induces stress that could confound fMRI reactivity (Fechir et al., 2010; Oei et al., 2012; Ossewaarde et al., 2011; Qin et al., 2012). One recent study of awake macaques utilized a restraint system including a customized silicone rubber head mold to maintain head stability, and demonstrated that high quality fMRI data can be obtained from macaques without the use of surgical implants or anesthesia (Murnane and Howell, 2010). The study's acclimation procedures were successful in limiting subject motion during scans while resulting in cortisol levels that remained stable for 2-hour restraint periods, suggesting that restraint did not induce potentially confounding levels of stress (Murnane and Howell, 2010).

We sought to assess whether a variation on that method could be useful for acquiring fMRI scans in cynomolgus monkeys. Specifically, we tested a commercially available restraint system using ear bars for head positioning (requiring subject sedation for initial positioning), and acquired fMRI scans post-anesthesia in alert monkeys. We assessed these procedures to determine whether they enable studies of fMRI effects of opioid agonists.

We studied two potent, high-efficacy opioid agonists with selectivity for mu (fentanyl) or kappa (U69,593) receptors (Negus et al., 1993). Mu and kappa receptors comprise two of the three opioid receptor subtypes that have been pharmacologically characterized (Martin et al., 1976; Lord et al., 1977; Kieffer, 1995). These receptors have different neuroanatomical distributions (Mansour et al., 1988; Mansour et al., 1994) and their activation produces qualitatively different neurochemical and electrophysiological effects (Spanagel et al., 1990; Ford et al., 2006). Mu and kappa agonists also produce distinct profiles of behavioral effects related both to clinically desirable endpoints (e.g. analgesia) and to undesirable effects (e.g. abuse potential) (Woods and Winger, 1987; Dykstra and Massie, 1988; Negus et al., 1993; Gutstein and Akil, 2005; Negus et al., 2008). In macaques, for example, both fentanyl and U69,593 produce thermal antinociception and disruption of operant responding maintained by food reinforcement (Negus et al., 1993; Negus et al., 2004), but these drugs have distinct discriminative stimulus effects (Butelman et al., 2010), and fentanyl is self-administered whereas U69,593 is not (Negus et al., 2008). Moreover, the antinociceptive and reinforcing effects of fentanyl in macaques are increased and decreased, respectively, by addition of U69,593 (Negus et al., 2008). Thus, mu-kappa opioid drug combinations might retain therapeutically useful antinociceptive effects while having reduced abuse liability, and might therefore help address the current epidemic of prescription painkiller abuse (Editorial, Lancet, 2011). We hypothesized that fentanyl and U69,593 would induce neuroanatomically distinct effects in reward-related brain areas, caudate nucleus and nucleus accumbens. To evaluate the degree to which restraint and scanning might elicit stress responses that could influence fMRI effects, we also measured plasma levels of the stress hormones adrenocorticotrophic hormone (ACTH) and cortisol immediately before and after imaging sessions in subjects that had been conditioned to restraint as well as MRI scanner sights and sounds.

METHOD

Subjects

Three experimentally naive adult male cynomolgus monkeys (*Macaca fascicularis*) weighing 4.9–7.8 kg were used as subjects. The subjects were individually housed and water was freely available. Their diet consisted of PMI Feeds Jumbo monkey diet (7–8 biscuits/

day; PMI Feeds, Inc., St. Louis, MO) supplemented with fresh fruit twice daily. A 12 hr light/12 hr dark cycle was in effect (lights on from 7AM–7PM).

Animal maintenance and research were conducted in accordance with the guidelines provided by the NIH Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was monitored daily by the staff veterinarian or by a veterinary technician. Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study. Monkeys also had access to puzzle feeders, mirrors, and chew toys to provide environmental enrichment, and nature videotapes were played daily in the housing room.

Acclimation Procedures

Prior to scanning initiation, all monkeys were acclimated to a commercially available MRI-compatible restrainer (InsightMRI, Worcester, MA), which holds subjects in a prone, “sphinx” position. Acclimation sessions were conducted 2–3 days per week in a procedure room at the animal housing facility. For each session, monkeys initially were sedated with ketamine 5 mg/kg IM, xylazine 1 mg/kg IM, and atropine 0.04 mg/kg IM. Monkeys then were transferred to the procedure room and secured in the MRI-compatible restrainer. A temporary intravenous catheter was implanted in a saphenous vein, and a pulse oximetry probe was attached to the lip or ear to permit measurement of heart rate and oxygen saturation, SPO₂ (Vet/OX G2 Digital Pulse Oximeter, Dolphin Medical, Hawthorne, CA). A dose of yohimbine (0.11 mg/kg IV) then was administered to antagonize the xylazine, and monkeys then were allowed to awaken while positioned in the restrainer. Initially, each monkey was kept in the restrainer for only 5–10 min after yohimbine administration. During subsequent acclimation sessions, restraint duration was gradually increased until monkeys remained in the restrainer for at least 1 hr without evidence of movement or overt struggling. Heart rate and SPO₂ measures were monitored for stability. Juice reward was used during and after acclimation sessions to couple restraint with positive reinforcement. For the three monkeys used in this study, acclimation required 17–20 sessions over 6–8 weeks.

Scanning Procedures

Transport and scan setup procedures—Once acclimation was complete, scanning sessions were initiated. Pre-scan sedation procedures identical to those used for acclimation (described above) were used prior to each scan session to facilitate monkey transport from the animal holding facility to the MRI suite (~0.25 miles) via a dedicated animal transport van. During transport, monkeys were contained in a ventilated stainless steel transfer cage covered by a drape. Upon arrival at the MRI suite, monkeys were placed in the MRI-compatible restrainer, a temporary intravenous catheter was placed in a saphenous vein, and a nasal cannula was inserted into the animal's nostrils to permit monitoring of end tidal CO₂ (Magnitude 3155MVS MRI Patient Monitor, Invivo Research, Orlando, FL). A dose of yohimbine (0.11 mg/kg IV) then was administered to antagonize xylazine, and the restrainer containing the monkey was placed on the MRI scanner patient table. Scanning sessions were conducted no more than once per week in any monkey.

MRI procedures—All scans were acquired on a 3.0 Tesla Siemens Trio whole-body MRI scanner (Siemens Medical Solutions USA Inc., Malvern, PA) using the standard quadrature volume head coil, through which the MRI-compatible restrainer was inserted. Localizer and structural images were acquired after yohimbine administration while subjects regained consciousness. Localizer and high-resolution T1-weighted structural scans optimized for grey/white matter contrast (3D MP-RAGE, FOV=192 mm, Slices=128, Slice Thickness=1.0 mm, TR/TE= 2100/2.74 ms, TI=1100 ms, Scan time=8:59 min) were collected. Once

recovery was complete (1 hr after ketamine, 0.5 hr after yohimbine), functional MRI (fMRI) data were acquired using a single shot Gradient Echo EPI sequence (FOV=120mm, slices=30, TR/TE=2500/30ms, matrix=64×64, SLT=3mm). To reduce scanner drift due to gradient heating, initial “prewarming” EPI scans (50 shots; scan time =2:05 min) were acquired before data acquisition (720 shots, scan time=30 min). Ten minutes after the start of the fMRI acquisition, subjects received an intravenous injection through the saphenous vein catheter either of saline (for determination of ACTH and cortisol changes), the mu agonist fentanyl (3.2 µg/kg), or the kappa agonist U69,593 (3.2 µg/kg). Fentanyl and U69,593 were tested at equivalent doses because these two drugs are approximately equipotent to modify rates of operant responding (Negus et al., 1993; Negus et al., 2008), and the intravenous dose of 3.2 µg/kg was selected based on drug potencies in an assay of intravenous drug self-administration (i.e. 3.2 µg/kg fentanyl is 10-fold higher than the lowest unit dose of fentanyl to maintain self-administration; Negus et al., 2008). Imaging continued for an additional 20 minutes. After scan sessions, field maps were obtained for image-unwarping purposes (scan time=3:20 min). Total scan time for each study was approximately 1 hr.

Data analysis—Functional MRI data analysis was performed in BrainVoyagerQX 1.7.9 for the Macintosh. BOLD fMRI scans were preprocessed to remove any spikes due to scanner instability or impulsive motion using a median filter implemented in IDL (Research Systems, Inc., Boulder, CO), and then slice scan time corrected, motion corrected and smoothed temporally (10 s Gaussian kernel) and spatially (5 mm isotropic Gaussian kernel) in BrainVoyagerQX prior to analysis. All functional data sets were resampled to 3D and aligned with the high-resolution T1-weighted anatomical images, and corrected for slight differences in brain size and orientation in a manner analogous to Talairach transformation (anterior commissure-posterior commissure registration, piecewise linear interpolation to a standard brain size) applied in human studies. Data were screened and image sets with artifacts were eliminated: scans were repeated if there was excessive motion on any of the 6-axis motion parameters or uncorrectable scanner spiking or other major abnormalities. Functional MRI data were processed using a General Linear Model (GLM) by using a step-function predictor with a transition at the injection time convolved with a hemodynamic response function as a correlate with pharmacological changes. Eight confound predictors also were added—the 6-axis motion parameters determined by BrainVoyagerQX during motion correction to compensate for translational (X,Y, Z) and rotational (X,Y, Z) motion effects, a whole-brain time course and linear ramp to compensate for scanner drift, and a data-derived physiological noise regressor to compensate for respiratory noise derived from the residuals from an initial fit (Madsen and Lund, 2006). Based on known sites of opioid actions, the GLM was restricted to several brain regions including the bilateral anterior and caudal caudate nucleus (Cd), and the nucleus accumbens (NAc, Figure 1). To confirm the ROI analysis and evaluate the influence of study drugs on other brain areas, we conducted an exploratory post-hoc whole-brain analysis.

Physiological Measurements

Since fentanyl and U69,593 can affect cardiovascular and/or respiratory function, which if perturbed could alter fMRI results, we initially tested whether 3.2 µg/kg drug doses affected heart rate, SPO₂, or end tidal CO₂.

End tidal CO₂ data were collected during scans in 2 monkeys (the nasal cannula was too large to use in the third monkey). Pulse oximetry data on heart rate and SPO₂ were not collected in the scanner because cynomolgus monkeys have darkly pigmented skin that necessitates pulse oximetry probe locations (e.g., toes or lip) not compatible with the equipment used in this experiment. Accordingly, pulse oximetry studies were conducted in a

procedure room in the animal housing facility (the same room used for acclimation sessions). In those parallel studies, monkeys were sedated as before scanning sessions, transported to the procedure room in the animal housing facility, and placed in the same MRI-compatible restrainer used for scanning. A temporary IV catheter was implanted in a saphenous vein, and a pulse oximetry probe was attached to each subject's toe or lip. Yohimbine (0.11 mg/kg IV) was administered as for scanning sessions, and animals were allowed to awaken for 40 min. Heart rate and SPO₂ then were recorded every minute for 30 min (i.e., the duration of fMRI scans). The first 10 min served as the baseline period and then a single dose of fentanyl (3.2 µg/kg) or U69,593 (3.2 µg/kg) was administered IV via the saphenous vein catheter. The next 20 min served as the experimental period. Pulse oximetry studies were conducted no more often than once per week and only during weeks when a scanning session was not scheduled for that monkey.

Pulse oximetry data were analyzed by one-factor ANOVA using Prism 4 for Macintosh (GraphPad Software, San Diego, CA). Significance on the ANOVA test was followed by the Dunnett's post hoc test to assess differences from baseline. The criterion for significance was set a priori at $p < 0.05$. End tidal CO₂ data are included in the graph but were not submitted to statistical analysis because only two monkeys were studied, and data for some time points were missing for one monkey after U69,593 treatment.

Endocrine Measurements

To assess the impact of restraint in the MRI-compatible restrainer and scanner noise on stress levels, blood samples were collected only during saline sessions (to avoid potential effects of fentanyl or U69,593) immediately after initial sedation at the start of the scanning procedure (i.e. before transport, restraint and scanning) and again immediately after scans as described above, and plasma levels of the stress-related hormones ACTH and cortisol were determined. Plasma ACTH concentrations were measured in duplicate with a radioimmunoassay kit (Catalog # 24130; DiaSorin Inc.; Stillwater, MN). Assay sensitivity was 7.0 pg/ml, and the intra- and inter-assay coefficients of variation were 4.9 and 11.8%, respectively. Plasma cortisol concentrations were measured in duplicate using a coated-tube radioimmunoassay (Clinical Assays™ Catalog # CA1529; Diasorin Inc., Stillwater, MN). The cortisol assay sensitivity was 0.16 µg/dl, and the intra- and inter-assay coefficients of variation were 7.4 and 11.7%, respectively. For comparison, effects of MRI restraint were compared to historical data on effects of looser restraint commonly used for behavioral studies of nociception and opioid antinociception in macaque monkeys (Negus et al., 2004). Data were analyzed by t-tests using Prism 4 for Macintosh, and the criterion for significance was $p < 0.05$.

Drugs

Fentanyl HCl (provided by the National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile water, and U69,593 (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.5% lactic acid in sterile water. Other study drugs (ketamine, xylazine, atropine, and yohimbine) were commercially available veterinary formulations. Drugs were filtered (MillexGV 0.22µm filter, Millipore, Billerica, MA) for IV administration.

Results

Cardiovascular and Respiratory Effects—Neither fentanyl nor U69,593 at the 3.2 µg/kg dose tested affected heart rate, SPO₂, or end tidal CO₂ (Figure 2), consistent with previous studies (Nussmeier et al., 1991; Vivian et al., 1998).

Functional MRI Findings—In an analysis for opioid effect (including fentanyl and U69,593 data), we found fMRI activation in all Cd and NAc ROIs (False Discovery Rate

(FDR) corrected to $P < 0.05$). Relative to fentanyl, U69,593 induced greater activation in the left NAc (FDR corrected to $P < 0.05$, Figure 3, top panel, peak activation located at Talairach coordinates: $x = -8$, $y = 17$, $z = -5$, number of voxels: 273). By contrast, fentanyl induced greater activation in the left Cd versus U69,593 (FDR corrected to $P < 0.05$, Figure 3, top panel, peak activation marked by crosshairs: $x = -18$, $y = 11$, $z = 22$, number of voxels: 246). The exploratory whole-brain analysis was consistent with our ROI results and also revealed that fentanyl induced more bilateral amygdala BOLD activation than U69,593 (Figure 3, bottom panel, coronal image, arrows). In addition, fentanyl induced greater BOLD activation than U69,593 in anterior cingulate cortex, dorsolateral prefrontal cortex, and left cerebellar hemisphere (Figure 3, bottom panel, blue-green colors). By contrast, U69,593 induced greater BOLD activation than fentanyl in right orbitofrontal cortex, dorsal thalamus, supplementary motor and motor cortex, parietal cortex, and brainstem (Figure 3, bottom panel, orange-yellow colors).

Subject motion during fMRI Scans—Subject head motion during fMRI scans was minimal and did not differ between fentanyl and U69,593 scans (complete shot to shot translational and rotational motion data are shown online in Supplemental Materials). Maximum shot to shot translational motion ranged from 0.038 to 0.126 mm, less than 10% of the 1.875 mm voxel size, and maximum shot to shot rotational motion ranged from 0.055 to 0.198 degrees (Table 1). These peak shot to shot deviations were well within the range of deviations that can be managed by BrainVoyager motion correction algorithms.

Endocrine effects of restraint and MRI scanning—Figure 4 shows the effects of the scanning procedure on plasma levels of the stress-related hormones ACTH and cortisol. The procedure produced a trend toward increases in ACTH and cortisol, although data for ACTH could not be statistically analyzed because of missing data from one monkey, and data for cortisol did not reach the criterion for statistical significance. Historical data obtained from a separate group of male rhesus macaques undergoing standard restraint employed for behavioral nociception (nonimaging) studies documented comparable if not greater ACTH and cortisol level increases (Negus et al., 2004).

Discussion

We utilized a commercial restraint system in combination with an acclimation protocol to minimize motion and stress confounds in alert post-anesthetized macaques undergoing fMRI. Subject motion in this study was minimal (Table 1 and online Supplemental Materials) and within the range of motion parameters reported by Murnane and Howell (2010), who utilized a customized plastic head mold system to immobilize subject heads during scanning. Accordingly, we believe that head motion did not confound fMRI data. Restraint and scanning produced a nearly two-fold increase in plasma levels of both ACTH and cortisol. However, the effects were comparable to or smaller than increases induced by restraint used for behavioral studies of nociception in rhesus monkeys (Negus et al., 2004). Accordingly, we believe that stress and stress-induced ACTH and cortisol increases were no more of a confound in this fMRI study of opioid effects than they are in common behavioral assays of opioid effects.

We found that 3.2 ug/kg i.v. doses of the mu opioid agonist fentanyl and the kappa opioid agonist U69,593 induced BOLD fMRI activation in Cd and NAc regions of interest. This was anticipated because mu and kappa receptors are enriched in these brain areas in humans (Vonkeman et al., 1996; Voorn et al., 1996) and because both mu and kappa agonists substantially increase [35 S]GTP γ S binding in these areas in cynomolgus monkeys, reflecting agonist-induced receptor-mediated second messenger coupling (Sim-Selley et al., 1999).

When we compared fentanyl and U69,593 effects, we found that U69,593 induced significantly greater fMRI activation than fentanyl in left NAc, whereas in left Cd, fMRI activation after fentanyl was significantly greater than after U69,593. These differential effects could be in part a result of regional receptor density differences, as kappa opioid receptor densities are nearly 60% higher in primate (human) NAc than in dorsal Cd (Vonkeman et al., 1996), whereas mu receptor densities are about 30% higher than kappa densities in human dorsal Cd (Voorn et al., 1996), or of receptor occupancy differences during fMRI scans. However, pharmacodynamics studies have documented very similar time courses for i.v. fentanyl and U69,593 to induce prolactin increases and ptosis, respectively, with peak effects occurring within 5–15 minutes (Butelman et al., 2008; Butelman et al., 2012), suggesting that pharmacodynamics differences between these opioids are not substantial, and we observed no differences in fMRI time courses in our study.

The apparent laterality effect we detected when contrasting U69,593 to fentanyl BOLD fMRI responses, with differences between drugs detected only in the left NAc and left caudal Cd, could be an artifact of our relatively small sample size. However, there is reason to believe that the laterality effect is real and may reflect relative rewarding and aversive properties of fentanyl and U69,593. In this regard, kappa agonists including U69,593 and dynorphin analogs have been shown to be aversive in animals (Bals-Kubik et al., 1989; Mucha and Herz, 1985; Shippenberg and Herz, 1987; Todtenkopf et al., 2004) and in humans (Giuffra et al., 1993; Pande et al., 1996a,b; Pfeiffer et al., 1986; Ur et al., 1997). Although the NAc typically is considered a key structure in reward-related processes, it also plays a role in aversive processes, with NAc neuronal firing increasing during aversive states (for review, see Carlezon and Thomas, 2009). Appetitive and aversive conditioned stimuli exert asymmetric effects in rat nucleus accumbens, with these stimuli preferentially affecting right and left nucleus accumbens dopamine levels, respectively (Besson and Louilot, 1995). A recent study of affective stimulus processing concluded that negative stimulus processing by subcortical regions was left-lateralized (Beraha et al., 2012). Further, the neuroimaging literature includes a number of precedents in which left NAc is activated to a greater extent than right NAc during painful or aversive stimuli (Becerra et al., 2001; Gottfried et al., 2002; Zald and Pardo, 2002; Diaconescu et al., 2009; Cauda et al., 2011). Taken together, these findings suggest that relative to 3.2 ug/kg fentanyl, 3.2 ug/kg U69,593 may enhance left NAc activity in cynomolgus monkeys by engendering a more dysphoric response.

An exploratory post-hoc whole brain analysis confirmed the ROI findings and revealed drug differences in additional brain areas, including bilateral amygdala (Figure 3, bottom panel, arrows). The amygdala difference (fentanyl > U69,593, blue-green colors) is consistent with reports of mu opioid (remifentanyl) activation and kappa opioid (nalbuphine) inhibition of BOLD responses in human amygdala (Leppä et al., 2006; Gear et al., 2013). Drug differences also were detected in a number of other brain areas including frontal executive, limbic, and motor areas. Given the limited sample size of the current study, these findings should be interpreted with caution, although they merit further study as they could be a basis for relative differences in reward and aversion induced by these opioids, and for their differential abuse liabilities.

Limitations

There are several limitations to this study that merit discussion. First, although our sample size was similar to that often used for behavioral studies in macaques (e.g. Negus et al., 1993; Butelman et al., 2010) and for macaque neuroimaging studies (e.g., Zhang et al., 2000; Murnane and Howell, 2010; Ku et al., 2011; Chen et al., 2012), it was smaller than that typically used in human neuroimaging studies. This small sample size constrained the

scope of brain areas that could be examined, so a region-of-interest analysis was used to focus on striatal areas of particular interest. Using this analysis, the sample size was sufficient to document differential effects of fentanyl and U69,593. Second, because we only studied single dose levels of fentanyl and U69,593, we cannot draw comprehensive conclusions about brain activation patterns induced by these opioids, which would require studying complete dose-effect relationships for each drug. Third, although our subjects were alert during fMRI scans, they were not drug-free when study drugs were administered, so we cannot rule out possible interaction effects between the opioids we studied and residual levels of atropine, ketamine, xylazine, and yohimbine. For example, yohimbine has been shown to be aversive (File, 1986) and has been used as a stressor in opioid reinstatement paradigms (Zhou et al., in press), suggesting its presence could be a confounder when investigating some effects of opioid agonists. Similarly, there may be residual effects of other sedation drugs that limit what questions can be addressed with our post-anesthesia approach. Notwithstanding these limitations, we believe that our study both illustrates an alternative method for conducting fMRI studies in alert nonhuman primates, which confers some advantages over methods utilizing anesthetized nonhuman primates, and shows that this method may enable differentiation of fMRI responses for opioid agonists with different pharmacological specificities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Regions of interest (ROIs) selected for analysis. Shown in white squares are bilateral ROIs in the rostral caudate nucleus (RCd, 1000 mm³), nucleus accumbens (NAc, 343 mm³), and the caudal caudate nucleus (CCd, 1000 mm³). A = anterior, P = posterior, R = right, L = left.

Figure 2.

Effects of fentanyl (3.2 $\mu\text{g/kg}$ IV) and U69,593 (3.2 $\mu\text{g/kg}$ IV) on heart rate ($\circ$), oxygen saturation (SPO₂, $\diamond$), and end tidal CO₂ ($\triangle$). Abscissae: Time (min) after fentanyl or U69,593 administration. Open data points above “BL” show average baseline data collected during the 10 min period prior to fentanyl or U69,593 administration. Ordinates: Units of beats/min (heart rate) or mmHg (SPO₂ or end tidal CO₂). Heart rate and SPO₂ data were collected using pulse oximetry in all 3 subjects during experimental sessions held outside the scanner. End tidal CO₂ data were collected using a nasal cannula in two monkeys during fentanyl and U69,593 scanning sessions. For U69,593 end tidal CO₂ studies, data from 14–18 minutes after drug administration are shown for only one monkey, because the cannula was temporarily dislodged during this period in the other monkey. All points show means $\pm$ SEMs, and, where no error bars are visible, the error is within the scope of the point. Neither fentanyl nor U69,593 significantly altered heart rate or SPO₂ at any time after administration. Data for end tidal CO₂ were not submitted to statistical analysis because only two monkeys were studied, and data from one monkey were missing for from 14–18 min after treatment with U69,593.

Figure 3.

Top Panel: Functional MRI results showing the contrast 3.2 µg/kg U69,593 > 3.2 µg/kg fentanyl within pre-selected ROIs (FDR corrected to $P < 0.05$). In left NAc, U69,593 induced greater BOLD fMRI activation than fentanyl. In left caudal Cd, U69,593 induced less BOLD fMRI activation than fentanyl. The crosshairs denote the caudal Cd cluster demonstrating differential BOLD fMRI responses to fentanyl and U69,593 response. Bottom Panel: Post-hoc whole-brain analysis fMRI results showing the contrast 3.2 µg/kg U69,593 > 3.2 µg/kg fentanyl (FDR corrected to $P < 0.05$). The whole brain analysis confirmed results of the ROI analysis and also revealed a significant drug difference in amygdala (yellow arrows), with fentanyl inducing a greater BOLD effect than U69,593 (blue-green colors). Areas in which U69,593's BOLD effects were greater than fentanyl are shown in orange-yellow colors. Shown are sagittal (left column), axial (middle column), and coronal (right column) slices. A = anterior; P = posterior; R = right; L = left.

Figure 4.

Changes in plasma ACTH and cortisol levels in male macaque monkeys produced either by restraint and behavioral testing in a standard primate restraint chair (standard chair) or by restraint and fMRI scanning (fMRI chair). The left bars in each panel show effects of restraint in a standard primate-restraint chair for behavioral testing in fully acclimated rhesus macaques (N=4; Negus et al., 2004). The right bars in each panel show the effects of restraint during a saline MRI scan in cynomolgus macaques on ACTH (N=2) or cortisol (N=3) levels. Baseline measurements are shown in open bars and post-restraint measures are shown in closed bars. Bars represent means $\pm$ SEMs. *Asterisks indicate significantly different from baseline ($p < 0.05$) as indicated by paired t-test. Data for ACTH levels in fMRI study monkeys were not submitted to statistical analysis due to the small N. However, ACTH levels approximately doubled in both monkeys that were assessed, resulting in the approximate doubling of mean ACTH levels shown in the figure.

Table 1Absolute motion statistics, Means $\pm$ SEMs

Statistic	Measure	Fentanyl	U69,593
Maximum	X translation (mm)	0.0264 (0.0136)	0.0239 (0.0705)
	Y translation (mm)	0.0398 (0.0059)	0.0606 (0.0157)
	Z translation (mm)	0.0287 (0.0080)	0.0924 (0.0167)
	X rotation (deg)	0.0514 (0.0030)	0.0682 (0.0286)
	Y rotation (deg)	0.0500 (0.0344)	0.0398 (0.0142)
	Z rotation (deg)	0.0730 (0.0214)	0.0812 (0.0589)
Mean	X translation (mm)	0.00006 (0.00005)	0.00003 (0.00007)
	Y translation (mm)	0.00028 (0.00013)	0.00038 (0.00002)
	Z translation (mm)	0.00002 (0.00005)	0.00043 (0.00020)
	X rotation (deg)	0.00028 (0.00011)	0.00005 (0.00024)
	Y rotation (deg)	0.00018 (0.00012)	0.00012 (0.00006)
	Z rotation (deg)	0.00050 (0.00018)	0.00036 (0.00043)
Standard Deviation	X translation (mm)	0.0041 (0.0022)	0.0032 (0.0007)
	Y translation (mm)	0.0076 (0.0007)	0.0091 (0.0019)
	Z translation (mm)	0.0065 (0.0019)	0.0133 (0.0012)
	X rotation (deg)	0.0091 (0.0013)	0.0104 (0.0044)
	Y rotation (deg)	0.0068 (0.0041)	0.0058 (0.0025)
	Z rotation (deg)	0.0134 (0.0047)	0.0127 (0.0102)