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Ketamine-induced brain activation in awake female nonhuman primates: a translational functional imaging model

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Abstract

Rationale—There is significant interest in the NMDA-receptor antagonist ketamine due to its efficacy in treating depressive disorders and its induction of psychotic-like symptoms that make it a useful tool for modeling psychosis.

Objective—The present study extends the successful development of an apparatus and methodology to conduct pharmacological MRI studies in awake rhesus monkeys in order to evaluate the CNS effects of ketamine.

Methods—Functional MRI scans were conducted in four awake adult female rhesus monkeys during sub-anesthetic i.v. infusions of ketamine (0.345 mg/kg bolus followed by 0.256 mg/kg/hr constant infusion) with and without risperidone pretreatment (0.06mg/kg). Statistical parametric maps of ketamine-induced BOLD activation were obtained with appropriate GLM models incorporating motion and hemodynamics of ketamine infusion.

Results—Ketamine infusion induced and sustained robust BOLD activation in a number of cortical and subcortical regions, including the thalamus, cingulate gyrus, and supplementary motor area. Pretreatment with the antipsychotic drug risperidone markedly blunted ketamine-induced activation in many brain areas.

Conclusions—The results are remarkably similar to human imaging studies showing ketamine-induced BOLD activation in many of the same brain areas, and pretreatment with risperidone or another antipsychotic blunting the ketamine response to a similar extent. The strong concordance of the functional imaging data in humans with these results from nonhuman primates highlights the translational value of the model and provides an excellent avenue for future research examining the CNS effects of ketamine. This model may also be a useful tool for evaluating the efficacy of novel antipsychotic drugs.

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The authors declare no competing financial interests.
Introduction

Ketamine is a non-competitive N-methyl-D-aspartate glutamate receptor (NMDAR) antagonist that has become the focus of a great deal of recent research in disparate fields of psychiatry because of a number of remarkable properties. While commonly used medically as a general anesthetic (Rowland 2005), sub-anesthetic ketamine infusion produces schizophrenia-like symptoms in healthy humans (Krystal et al. 1994), generates a rapid antidepressant response in patients with treatment resistant depression (Berman et al. 2000), and shows efficacy in treating neuropathic pain (Schwartzman et al. 2009). This extraordinary profile of effects makes ketamine a key research target for improving the scientific understanding of schizophrenia and depression.

Interest in glutamatergic agents as potential treatments for schizophrenia is based primarily on the effects of NMDAR antagonists, and ketamine in particular, which acutely mimic the positive, negative, and cognitive symptoms of schizophrenia in healthy humans (Krystal et al. 2013; Olney and Farber 1995). All antipsychotic medications in current clinical use act primarily on the dopamine system (most are D2 antagonists) and are only efficacious for positive symptoms with little efficacy for alleviating the negative or cognitive symptoms (Miyamoto et al. 2005). Recently, research has shifted increasingly towards glutamatergic compounds (Stone 2011), however, thus far large clinical trials have been unsuccessful (Weiser et al. 2012). PET imaging has shown that acute administration of ketamine increases dopamine release in the striatum, and the extent of dopamine elevation in the ventral striatum correlates strongly with onset of positive symptoms (Vollenweider et al. 2000). Thus, acute ketamine challenge as a model for schizophrenia is consistent with evidence for the involvement of both the dopaminergic and glutamatergic systems (Frohlich and Van Horn 2014).

Pharmacological MRI (phMRI) has been used to identify the pattern of activation induced by sub-anesthetic doses of ketamine in human volunteers by measuring changes in blood oxygenation level dependent (BOLD) signal (De Simoni et al. 2013; Deakin et al. 2008; Doyle et al. 2013). These changes in BOLD signal correlate with the subjective effects of ketamine (Deakin et al. 2008) and this technique also has shown very good test-retest reliability (De Simoni et al. 2013). Thus, phMRI provides a reliable, in vivo imaging methodology for studying the whole-brain pharmacological effects of ketamine. Of particular relevance to schizophrenia, pretreatment with the antipsychotic risperidone has been shown to attenuate the subjective effects (Schmechtig et al. 2013), BOLD activation (Doyle et al. 2013), and functional changes (Joules et al. 2015) induced by ketamine. This is particularly interesting because while risperidone acts as an antagonist with high affinity for dopamine D2, 5-HT2a, and a number of other receptors (Meltzer and McGurk 1999), like other clinical antipsychotic compounds it does not interact directly with NMDARs.
Risperidone may provide a good benchmark for comparison of the effectiveness of novel antipsychotics to attenuate or reverse the CNS effects of ketamine, and evidence suggests that phMRI provides a unique tool for making this comparison.

Nonhuman primate (NHP) models offer distinct advantages for studying cognitive dysfunction and psychopathology (Phillips et al. 2014). Both schizophrenia (Lewis and Lieberman 2000) and depression (Mayberg 2003) are characterized in part by altered processing in prefrontal cortex (PFC) and limbic circuits and NHPs represent an excellent animal model because their behavioral repertoires are sophisticated and their PFC is closely aligned with humans (Preuss 1995). The present study extends the successful development of an apparatus and methodology to conduct phMRI studies in conscious rhesus monkeys (Murnane et al. 2015; Murnane and Howell 2010) in order to evaluate the CNS effects of antipsychotics. The first aim of this study was to validate NHPs as a model for studying the CNS effects of ketamine by using phMRI in awake rhesus monkeys to examine BOLD activation/deactivation profile of ketamine against time. To evaluate the translational utility of this model the whole brain profile is presented along with in depth analysis of particular regions that are known to be involved in the psychopathology of both schizophrenia and depression. A second aim of the study was to evaluate the interaction of ketamine with the clinical antipsychotic drug risperidone. The results obtained provide a sound metric for evaluating novel antipsychotics.

Materials and Methods

Subjects

The subjects were four adult female rhesus monkeys (Macaca mulatta) weighing 5.4–7.7 kg. All subjects were initially naïve to any experimental drugs and all underwent the same experiments and served as their own controls to increase statistical power and reduce the number of subjects necessary to complete the scientific objectives of the study. Clinical veterinarians at the Yerkes Center use ketamine for chemical restraint during animal surveys so all subjects had previous exposure to the drug. The endocrine status of the female subjects was not monitored. Animal use procedures were in strict accordance with the National Institutes of Health’s “Guide for the Care and Use of Laboratory Animals” and were approved by the Institutional Animal Care and Use Committee of Emory University.

Surgery

Subjects were surgically implanted with chronic indwelling venous catheters as described previously (Howell and Fantegrossi 2009). Briefly, under isoflurane anesthesia, a silicone catheter was implanted in the femoral vein and was passed to the level of the vena cava. The distal end was attached to a titanium port located subcutaneously in the mid-scapular region.

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. Subjects were first acclimated to transportation within the frame of the custom apparatus and being brought to the laboratory for 30-minute sessions three times per week. Gradually 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. Sessions were then reduced to one per week and the duration of immobilization within a session was gradually increased from 30 minutes to 2 hours. Audio recordings of several MR pulse sequences were played during these sessions to acclimate the subjects to the noises produced by the MR scanner. Finally, several full mock scanning sessions were undertaken where the subjects were immobilized in the apparatus and placed within the bore of the Yerkes Imaging Center MRI scanner to expose the subject to the scanner environment prior to the collection of experimental data.

**MRI data acquisition**

The apparatus and animal habituation protocol have been described in detail previously (Murnane and Howell 2010). Scans were conducted in a Siemens (Siemens Healthcare, Erlangen, Germany) Trio 3 Tesla magnet. The monkeys lay prone in a custom-built restraint cradle optimized for acquiring phMRI data from fully-conscious rhesus monkeys (Murnane and Howell 2010) and attached to a NHP head coil (RAPID MR International, LLC). Heart rate, oxygen saturation and expired gases were continuously monitored to ensure the safety of the animals inside the scanner. In each scanning session, BOLD sensitive phMRI images were collected utilizing a whole-brain gradient echo single-shot echo planar imaging (EPI) sequence. The scan parameters for this sequence were Repetition Time (TR) = 3000ms; Echo Time (TE) = 32ms; Flip Angle (FA) = 90°; Field of View (FOV) = 96cm, 1.5mm × 1.5mm in-plane resolution with 42 coronal slices covering the whole-brain; slice thickness = 1.5mm; 1100 measurements). Field inhomogeneities were mapped using a standard Siemens dual gradient echo based field mapping sequence for later correction of any EPI image distortions. A T1-weighted (T1w) a 3-dimensional (3D) magnetization prepared rapid gradient echo (MPRAGE) sequence (TR = 2300 ms; TE = 2.7ms; Inversion Time (TI)=800 ms; FA = 8°; FOV = 96cm; 1.5mm × 1.5mm × 1.5mm resolution) was also acquired to assist in co-registration of the EPI time-series to the high-resolution T1w anatomic acquired in a different session. For each monkey, a set of 7 high resolution T1w anatomic scans were acquired in a separate scanning session with a 3D MPRAGE sequence with scan parameters: TR = TR = 2300 ms; TE = 3.4ms; TI = 800ms; FA = 8°; FOV = 128cm; 0.5mm ×0.5mm × 0.5mm resolution. The 7 high-resolution anatomic images were averaged together to yield a final 3D T1w anatomic image with high SNR and resolution for anatomic reference.

**Drug infusion protocols**

Each subject underwent two 55-min pharmacological MRI scans in separate scanning sessions:

1. One minute baseline followed by a 1 minute bolus i.v. infusion of 0.345 mg/kg of ketamine followed by 53 minute continuous infusion of 0.256 mg/kg/hr ketamine;

2. Same as (1) but with risperidone (0.06 mg/kg, i.v.) administered 1 hour prior to the MRI session;

Note the infusion and dosing parameters were determined empirically from pharmacokinetic evaluations in order to achieve steady-state plasma levels of ketamine at approximately 100 ng/mL. Further, in order to ensure that expected drug levels were achieved during scanning,
blood samples were acquired immediately following each scan. Drug levels were determined from heparinized plasma by Tandem Labs using gas chromatography and mass spectrometry.

The effects of ketamine under dosing conditions identical to those employed during MRI scans were evaluated while subjects were seated in a standard primate chair in the behavioral laboratory. Subjects were fully conscious and responsive to auditory and tactile stimulation.

**Pharmacological MRI data analysis**

**Preprocessing and spatial normalization**—MRI data analysis was conducted with AFNI (Cox 1996) and FSL (Smith et al. 2004) software packages. The phMRI time-series images were first corrected for distortions introduced by magnetic field inhomogeneities (estimated by the gradient echo field map). Voxel time-series in the phMRI dataset were temporally shifted to account for differences in slice acquisition times. The 3D scan volumes were then registered to a base volume to account for motion.

The skull-stripped averaged high-resolution T1w anatomic structural image of subject RNE13 was chosen as the template brain for the four-subject dataset. RNE13’s brain was chosen due to its image quality and symmetry being the best among the four subjects scanned. Each subject’s averaged high-resolution high SNR T1w anatomic was registered to this template T1w dataset. For each subject, the low-resolution T1w anatomic acquired during the drug–infusion scan session was aligned to the template brain via the averaged high-resolution high SNR T1w anatomic acquired in a separate scanning session through image registration tools available in FSL. The motion-corrected EPI drug-infusion phMRI time-series was aligned to the low-resolution T1w anatomic acquired in the same session with a rigid registration algorithm and then aligned to the template brain through the warp calculated during the low-resolution T1w anatomic to template brain registration described above. The resultant EPI time-series were spatially smoothed with a full-width at half-maximum (FWHM) = 3mm isotropic Gaussian filter. The brain activation to each drug paradigm was assessed using this preprocessed phMRI time-series as described below.

**Pharmacological MRI data quality control**—The phMRI image time-series data were examined for large motions defined as more than 2mm displacement. Due to large motion exhibited by the NHPs during the end of a number of drug infusion scans, all the phMRI time-series were curtailed to 40 minutes. More than 2mm motion in more than 10% of the phMRI volumes within the first 40 minutes of a dataset was considered unusable. None of the scans acquired for these experiments exceeded this threshold and thus no scanning sessions were discarded or repeated.

**Activation mapping**—For each subject, brain activation to ketamine was estimated by adapting a ketamine phMRI model described in the literature (De Simoni et al. 2013) to observed region of interest (ROI) averaged ketamine infusion phMRI time-series in *a priori* ROIs (see below) across subjects. These drug infusion ROI-averaged phMRI responses were sustained (see Figure 4) throughout the duration of the drug infusion (after an initial ramp-up period of 2–3 minutes) in a number of ROIs (as opposed to the transient response shown
in (De Simoni et al. 2013)). To maintain a degree of translatability with human ketamine infusion studies and as well as to maintain consistency with observed sustained a priori ROI-averaged drug infusion responses, we employed the signal model in De Simoni et al. 2013 as a gamma-variate kernel (with parameters adjusted to achieve a time to peak of 2 minutes) with which we convolved the drug infusion time-course to obtain a reference phMRI signal time series, \( S(t) \) (normalized to a peak value = 1) for our general linear model.

\[
S(t) = \left( \frac{t}{t_{\text{max}}} \right)^b e^{b(t - t_{\text{max}})} \odot D(t), \quad \text{Eq. (1)}
\]

where \( S(t) \) is the modeled phMRI BOLD signal, \( D(t) \) is the drug infusion protocol, \( b \) is a shape parameter with value \( b = 0.01 \) and \( t_{\text{max}} \) is the time-to-peak amplitude. We set \( t_{\text{max}} = 120 \text{ sec} \) (as opposed to 240 sec in humans estimated by De Simoni et al.) due to the smaller brain size of NHPs compared with humans, as well as to maintain consistency with the observed signal in a priori brain ROIs. The reference vector \( S(t) \) (normalized to a peak value = 1), better represented the observed phMRI signal observed in this study.

The voxel phMRI time-series was modeled as

\[
y_i = \beta_i \times S_i + \sum_k \hat{a}_k M_i^k + \varepsilon_i, \quad \text{Eq. (2)}
\]

where \( y_i \) is the voxel intensity at \( i^{th} \) time-point, \( S_i \) is the \( i^{th} \) time-point of the reference signal time-course, \( M_i^k \) is the value of the \( k^{th} \) motion parameter (\( k = 1 \) to 6 corresponding to 6 degrees of rigid body motion) for the \( i^{th} \) volume, \( \beta \) is the amplitude of phMRI signal (Eq. (1)) and \( \hat{a}_k \) denotes the proportion of voxel phMRI time-series that is related to the \( M_i^k \) motion parameter. \( \beta \) and \( \hat{a}_k \) are obtained from Eq. (2) through least squares linear regression. Volumes exhibiting more than 2mm motion were discarded from analysis.

**Accounting for variations in durations of drug response in different brain regions**—The drug infusion model employed assumes that the brain activation assessed by the phMRI BOLD response is persistent throughout the drug infusion periods. In order to account for brain regions which (unlike the a priori ROIs) may lose responsiveness to drug infusion after varying time durations, the general linear regression model (GLM) described in Eq. (2) was fitted for 22 different periods of drug infusion: 6 min to 40 min (in steps of 2 min) as well as odd multiples of 5 from 5 min to 35 min. This procedure captures the complete drug response profile of all voxels in the brain. It is an adaptation of a well-established unconstrained BOLD response estimation technique in long block fMRI paradigms (Cato et al. 2004; McGregor et al. 2015; Moffett et al. 2015) to the constrained phMRI BOLD response estimation employed in this study. The only difference being instead of simultaneously estimating the amplitude of the BOLD response at 22 different time points after the start of infusion in one GLM, 22 separate GLMs were run and the amplitude of the BOLD response and its associated t-statistic were computed for each of the 22 infusion periods. This amplitude t-statistic is a standardized quantity that accounts for both the intensity of estimated amplitude as well as the error in the estimate. It was expected that voxels exhibiting sustained phMRI response throughout the 40 minute duration of the
drug infusion modeled would exhibit strong and significant GLM-estimate amplitude t-statistic in GLM analyses corresponding to all 22 infusion periods, and voxels with transient phMRI signal responses will exhibit loss of significance in the GLMs corresponding to the infusion periods where the voxel signal departs from the modeled sustained response. The summary statistics combining all the GLM-estimate amplitude t-statistics described below will express the strength of activation across the 40 minute drug infusion duration.

**Group-level drug treatment-induced whole brain activation maps**—The dose response profile for each voxel using the above procedure is a 22-point time course comprised of amplitudes (expressed in standardized t-scores) of phMRI BOLD response at each of 22 different modeled periods of drug infusion. For each voxel, the area under the curve (AUC) of the dose response curve (Cato et al. 2004; McGregor et al. 2015; Moffett et al. 2015), expressed as the mean of the 22-point GLM-estimate amplitude t-statistic time-course was employed to assess brain activation to each drug treatment paradigm (ketamine and ketamine after risperidone pretreatment). Group-level activation maps for ketamine and ketamine after risperidone pretreatment were obtained through 1-sample t-test on the individual subject whole-brain voxel-wise AUC maps.

**Between-treatment differences in whole brain activation**—The group-level differences in brain activation between different drug treatment conditions were obtained through a paired 2-sample t-test between the individual subject AUC maps of the two conditions. The drug treatment related brain activation and between-treatment t-test maps were clustered and the significance of activations accounting for multiple comparisons were derived by means of Monte Carlo simulation of the process of image generation, spatial correlation of voxels, intensity thresholding, masking and cluster identification (Forman et al. 1995) through the 3dClustSim program implemented in AFNI software. Specifically all the p-values reported in the Results section pertaining to whole-brain activation maps are reported at a multiple comparisons corrected significance of p < 0.05. To achieve this significance, the activation and contrast maps were masked with a whole-brain EPI mask, and activation intensity thresholded at individual voxel-level test-statistic threshold of p < 0.05, and clustered with a minimum volume threshold of 94 voxels arrived at by performing the Monte-Carlo simulation mentioned above through the 3dClustSim program.

**Non-parametric analysis**—Finally, due to the small sample size (N= 4), voxelwise Wilcoxon signed rank test was also performed to assess between-treatment differences in brain activation, as a non-parametric equivalent of the paired t-test. Multiple comparison corrected significance was obtained through the method described above (Forman et al. 1995).

**Analysis of activation in specific a priori brain ROIs**—In order to examine the temporal evolution of the drug response in selected a priori brain ROIs pre-defined ROIs were manually delineated by a single highly experienced rater (Dr. Urushino) in direct reference to the Paxinos rhesus monkey brain atlas (Paxinos et al. 2000). ROIs were drawn on the high resolution anatomical images to include every voxel fully contained within the following brain areas: anterior, mid, and posterior cingulate cortex (ACC, MCC, and PCC),
dorsolateral prefrontal cortex (dlPFC), medial prefrontal cortex (mPFC), orbital frontal cortex (OFC), supplementary motor area (SMA), superior frontal cortex, superior temporal gyrus (STG), caudate, putamen, thalamus, and amygdala. These areas have been shown to activate during ketamine infusion (De Simoni et al. 2013; Deakin et al. 2008) or be involved in cortico-limbic processing (Haber 2003; Johansen-Berg et al. 2008). All ROIs were drawn bilaterally, however amygdala, caudate, and STG were later separated into left and right hemispheres because significant clusters of ketamine activation or attenuation of ketamine activation by risperidone were seen in only one hemisphere. For each ROI, significant ketamine-induced brain activation and differences between drug treatment conditions were assessed by masking the group-level activation and t-contrast maps by each ROI. These masked statistical parametric maps were thresholded at individual voxel-level p < 0.05 and clustered with a minimum volume threshold corresponding to the extant needed to be significant at a multiple comparisons (incorporating both voxels in each ROI and the number of ROIs) corrected significance level of p < 0.05 obtained through Monte Carlo simulations (Forman et al. 1995) implemented with the 3dClustSim program. Multiple comparisons correction for the number of ROIs was conducted with the bonferroni method. Since the number of ROIs were N = 16 this amounted to setting the cluster-volume threshold for clusters within each ROI at the level needed to obtain a cluster-level p < 0.003.

**phMRI BOLD drug response time-course in specific a priori brain ROIs**—ROI-average BOLD dose response curves were obtained for each subject for each ROI by averaging the voxel time-series (after regressing out signal proportion to motion parameters (Bullmore et al. 1999)). The individual subject BOLD dose response curves for each ROI were averaged together to form group-averaged BOLD drug response time-course for that ROI.

**Clinical Ratings of Behavior**

The effects of risperidone (0.06 mg/kg) on clinical ratings of behavior (Casey et al. 2001) were evaluated while subjects were seated in a standard primate chair in the behavioral laboratory. Behaviors were scored before and 15, 30, 45, 60, 75, 90, 105, 120 min after risperidone (0.06 mg/kg) administration. Behaviors rated included: eye blinking (number/30 s), tongue protrusions (number/30 s), oral facial dyskinesias (involuntary repetitive movements of the mouth and face, number/30 s), chewing (number/30 s), sedation (0–3), stereotypy (constant repetition of certain meaningless gestures or movements, 0–3), dystonia of the head and neck (impairment of muscle tone, 0–3), trunk (0–3), upper limbs (0–3), and lower limbs (0–3); bradykinesia (0–3), tremor (involuntary trembling movement, 0–3), salivation (0–3), locomotor activity (−3 to 0, 0–3), and reactivity (−3 to 0, 0–3). The absolute value of the score corresponds to 0 = normal behavior or symptoms not present; 1 = mild (behavior occasionally present); 2 = moderate (behavior regularly present but interrupted); and 3 = significant (behavior continuously present). Eye blinking, tongue protrusions, oral facial dyskinesias, and chewing scores are the average number per 30 s of three consecutive 30-s rating periods. Reactivity responses to external stimuli and locomotor activity were scored with a scale range of −3 to +3. Reactivity responses were assessed as the reaction to the observer standing approximately 3 feet from the animal and moving one hand toward the animal over a 15-inch distance as if to touch the animal. This hand movement should evoke...
a consistent threat display toward the observer (0 = subject is alert, exhibits normal vigilance and responsiveness to the stimulus; −1 = slight decrease in responsiveness; −2 = moderate decrease in responsiveness to the stimulus; −3 = no response to stimulus at all, +1 = slight increase in responsiveness to the stimulus, +2 = moderate increase in reaction to the stimulus, +3 = marked exaggerated response to the stimulus). Locomotor responses were assessed as follows: 0 = subject is alert, exhibits normal amount of activity, −1 = slight decrease in activity, −2 = moderate decrease in activity, −3 = significant decrease in activity, +1 = slight increase in activity, +2 = moderate increase in activity, +3 = significant increase in activity.

Results

Blood plasma drug levels

Blood samples taken immediately after scanning were analyzed for plasma levels of ketamine and risperidone (Table 1). Measurements indicate a mean plasma ketamine concentration of 104 ± 8 ng/mL following the ketamine infusion phMRI scan (Scan 1 in Table 1) and 96 ± 24 ng/mL following the ketamine infusion phMRI scan conducted after risperidone pretreatment (Scan 2 in Table 1). The mean plasma risperidone concentration following Scan 2 was 1.7 ± 0.4 ng/mL. All plasma drug levels were within the expected range and ketamine dosage was sufficient to produce a significant drug effect in every scan.

Ketamine-induced BOLD activation

Ketamine infusion induced significant (p < 0.05) activation in an extensive number of brain areas as shown in Figure 1 and summarized in Table 2. Areas of significant activation include the superior frontal gyrus, supplementary motor area (SMA), anterior cingulate cortex (ACC), insula, superior temporal gyrus (STG), precuneus, primary somatosensory cortex (S1), thalamus and basal ganglia, and cerebellum. The strongest activation to ketamine was observed in the cingulate gyrus, SMA, and thalamus. There were no areas where significant deactivation was observed.

Reduction in Ketamine-induced Activation by Risperidone pretreatment

Pretreatment with Risperidone (0.06 mg/kg) an hour prior to infusion of ketamine reduced both the magnitude and extent of ketamine-induced brain activation. However, significant (p<0.05) residual brain activation to ketamine infusion remained in a number of areas (shown in Figure 2 and summarized in Table 2). This decrease in activation is clearly seen by comparing Figure 2 with Figure 1 (which use the same activation color scale, and slices to map ketamine activation) and is highlighted further by Figure 3 which shows areas where risperidone pretreatment caused a significant (p<0.05) change in ketamine-induced brain activation. Risperidone pretreatment significantly reduced (shown in blue in Figure 3) ketamine-induced brain activation in the SMA, cingulate gyrus, superior frontal gyrus and left hemisphere thalamus, caudate and putamen. No brain areas exhibited a significantly greater response to ketamine following risperidone pretreatment. The results from the non-parametric Wilcoxon signed rank test analysis closely resembled the paired t-test analysis results (see Online Resource 1).
Ketamine-induced activation and the effect of risperidone pretreatment in a priori ROIs

Specific effects of ketamine infusion within a priori ROIs that contained clusters of significant ketamine-induced activation following bonferroni correction for multiple comparisons are shown in Table 3. These a priori ROIs were selected either because they showed a strong response to ketamine in the literature or because of the importance of the region within the cortico-limbic circuit. As seen in the table, within these specific ROIs the strongest ketamine-induced activation occurred in the ACC, MCC, thalamus, caudate, putamen, and precuneus. Risperidone pretreatment reduced the response to ketamine in both magnitude and extent in almost every ROI with the largest effects seen in the ACC, anterior STG, thalamus, caudate, and putamen. All ROIs are bilateral except for amygdala, caudate, and STG where left and right hemispheres are considered separately because significant clusters of ketamine activation or attenuation of ketamine activation by risperidone were seen in only one hemisphere.

ROI-averaged Group-mean Time Courses

Figure 4 shows the ROI-average group-mean drug time-course plots for the anterior cingulate cortex (ACC), thalamus, orbitofrontal cortex (OFC), and the left caudate nucleus. The percentage change in BOLD signal during ketamine infusion is plotted over time. Each of the selected areas exhibit robust activation to ketamine infusion. The drug time-course curves indicate that ketamine-induced activation in these areas becomes evident within five minutes of the initial bolus and remains constant through the duration of the infusion. Pretreatment with risperidone blunts the ketamine response over the full time course in each of these regions. The low between-subject variation (indicated by the relatively small error bars) suggests that both ketamine-induced activation and the blunting of the ketamine response by risperidone are highly consistent across subjects over the full course of the infusion. Time course plots for some additional ROIs are shown in Online Resource 2.

Clinical Ratings of Behavior

Behavioral ethograms were evaluated in all 4 subjects at baseline and following acute risperidone challenge. Risperidone (0.06 mg/kg, i.v.) induced marked effects on sedation, motor activity and reactivity as shown in the ethograms reported in Table 4.

Discussion

The results of this phMRI study in fully-conscious NHPs show robust BOLD activation induced by a sub-anesthetic ketamine dosing regimen. These data are in excellent agreement with human phMRI imaging studies (De Simoni et al. 2013; Deakin et al. 2008; Doyle et al. 2013) which consistently show a pattern of brain activation following ketamine administration that is strikingly similar to the NHP data presented here. De Simoni et al. (2013) reported the most robust ketamine response in midline regions including the ACC, PCC, paracingulate gyrus, SMA, precuneus, cerebellum, thalamus, and brainstem. The ketamine response in the NHPs exhibited a nearly identical profile with each of those regions becoming significantly activated and the cingulate, SMA, and thalamus showing the greatest response. The excellent concordance between the human and NHP data suggests
that phMRI in conscious NHPs provides a reliable and translational animal model for investigating the CNS effects of subanesthetic ketamine.

Interestingly, the subgenual cingulate was one brain region in which the NHP data did not replicate the human results. This region is of particular interest because it appears to be located at a critical juncture of the cortico-limbic pathway (Mayberg 2003) and could play an important role in mediating the antidepressant effects of ketamine (Mayberg et al. 2005). No significant response to ketamine was detected in this region despite previous studies showing a significant ketamine-induced deactivation in humans. However, De Simoni et al. (2013) found that the reduction of BOLD signal with ketamine in the subgenual cingulate had a relatively low effect size and was much less reliable than BOLD increases observed in other regions such as the ACC, PCC, and thalamus. Indeed, with only four subjects, the present study may have been underpowered for detecting a deactivation in the subgenual cingulate. Thus the lack of an observed deactivation in the subgenual cingulate does not necessarily represent a meaningful difference between the human ketamine response profile and that of NHPs.

Further evidence for the utility of this NHP ketamine model for evaluating antipsychotic drugs is provided by the results of pretreatment with the standard clinical antipsychotic risperidone. While risperidone pretreatment significantly attenuated the ketamine response, strong residual ketamine-induced activation was still observed. This result is in excellent agreement with the human data and closely mirrors the findings from Doyle et al. (2013). Overall the similarity observed between human and NHP data in the results of risperidone pretreatment on the ketamine response lends further validity to the use of the NHP model and also provides both a proof of concept and a sound benchmark for using this model to test novel antipsychotics.

A number of sex differences have been associated with both schizophrenia (Abel et al. 2010) and depression (Young and Korszun 2010), so it should be noted that all four subjects in this study were female. This may be considered an advantage of the present study as all of the human ketamine phMRI data in previous studies were collected only in men. Given that males have been shown to be more vulnerable to the cognitive effects of ketamine than females (Morgan et al. 2006), it is critical to evaluate drug effects in both sexes.

**Limitations**

One limitation of the present study is that the menstrual cycles of the female subjects were not monitored. While it is possible that the endocrine status of the subjects affected our results, most of the evidence in the literature suggests that any effects should be minimal at most. A review of the effects of ovariectomy on NMDARs found no effects in brain regions with the exception of the hippocampus (Cyr et al. 2001). Further, a recent study found no effect of ovariectomy on the disruption of pre-pulse inhibition by ketamine in rats (van den Buuse et al. 2015).

Another limitation which is general to all awake NHP MRI studies is potential for motion artifacts to influence the results. In this study, the NHPs were trained extensively to tolerate the MRI scanner environment. In terms of data analysis, we regressed out phMRI signal.
proportional to movement parameters and furthered censored volumes of the phMRI signal time course during which the NHPs exhibited more than 2 mm motion. However residual motion artifacts may in part contribute to the increased variability seen in ROI-averaged ketamine infusion-induced phMRI response time-courses seen in Figure 4 when compared to those from the ketamine infusion scans conducted after pre-treatment with risperidone. Interestingly, the results from blood samples showed that between-subject variance in plasma ketamine levels was greater in risperidone pretreatment condition than ketamine-alone scans. These seemingly contradictory results can be explained in part by the observation that while all subjects remained awake and alert during the risperidone pretreatment scans, they exhibited significantly less motion compared to the ketamine-alone scans presumably due to the sedative effects of risperidone seen at clinical doses of the kind administered in this study. The reduction in amount of movement may explain the reduced variability in risperidone pretreatment ROI-averaged time courses seen in Figure 4. However, it must be noted that even given the increased variability in the ketamine-alone condition significant widespread activation in expected brain regions was observed, and statistically significant attenuation in phMRI response to ketamine was observed after pre-treatment with risperidone as expected.

No experiments were done to test the BOLD response to risperidone alone. While this may be an interesting future direction, to our knowledge no phMRI testing has examined the effects of risperidone (or other antipsychotic) alone, only the interaction with a task (Bolstad et al. 2015) or a psychotomimetic such as ketamine (Doyle et al. 2013).

As discussed previously, unlike previous experiments in humans, the NHP data shows no deactivation was observed in the subgenual cingulate in response to ketamine. This may represent a true species difference and could be a limitation to the translational validity of the model. However, data for this study was collected in only 4 subjects. While the effects shown are robust and appear to be highly consistent with previous studies in humans in the remainder of the brain, the small sample size limits the conclusions that can be drawn from these results.

**Future directions**

Pharmacological MRI in NHPs shows great promise as a translational model for the CNS effects of ketamine. Future studies should employ this model to examine drug interactions with the ketamine response as a method to further the understanding of the effects of this remarkable compound on the CNS. As a model for schizophrenia, phMRI in NHPs may be particularly effective for evaluating novel antipsychotics. The antipsychotic drugs currently in clinical use tend to feature moderate to severe side effects (Kim et al. 2007) and have shown little efficacy for treating the negative and cognitive symptoms of schizophrenia (Strous et al. 2003). Novel compounds that show similar efficacy to current antipsychotics could potentially be superior therapeutics if they simply exhibit a reduced side effect profile or provide effective treatment for the negative symptoms of schizophrenia.

Important questions regarding the efficacy of ketamine for treating depression remain unanswered. Randomized controlled trials have shown that a single I.V. infusion of ketamine can reliably generate a rapid antidepressant response in patients with treatment
resistant depression (Aan Het Rot et al. 2012), yet the mechanisms underlying the antidepressant effects of ketamine remain incompletely understood. Increased excitatory glutamatergic signaling may be necessary for the antidepressant effects of ketamine (Maeng et al. 2008) as well as synaptic strengthening in prefrontal (Li et al. 2010) and limbic (Autry et al. 2011) regions. Indeed, the increased neural plasticity induced by ketamine in cortico-limbic circuits may be a critical factor underlying its efficacy for treating both depression (Thompson et al. 2015) and neuropathic pain (Doan et al. 2015). As a highly translational model, phMRI in awake NHPs provides a valuable tool for studying the broad CNS effects induced by ketamine.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgements**

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**References**


Joules R, Doyle OM, Schwarz AJ, O'Daly OG, Brammer M, Williams SC, Mehta MA. Ketamine induces a robust whole-brain connectivity pattern that can be differentially modulated by drugs of different mechanism and clinical profile. Psychopharmacology (Berl). 2015


Fig. 1.
Group-level brain activation to ketamine: Map of 1-sample group t-test on individual subject dose-response AUC (see text) shows areas of significant brain activation during ketamine infusion. Ketamine induces extensive activation throughout the brain. Cluster-level multiple comparison corrected $p < 0.05$. Slices progress from posterior (top-left) to anterior (bottom-right)
Fig. 2.
Group-level brain activation to ketamine after risperidone pretreatment: Map of 1-sample group t-test on individual subject dose-response AUC (see text) shows areas of significant brain activation during ketamine infusion. Ketamine induced brain activation is still evident, but is considerably less extensive following risperidone pretreatment. Cluster-level multiple comparison corrected p < 0.05. Slices progress from posterior (top-left) to anterior (bottom-right)
Fig. 3.
Group-level difference in brain activation to ketamine after pretreatment with the anti-psychotic risperidone. Map of between-condition paired t-test shows areas where risperidone pretreatment significantly reduced brain activation to ketamine. Cluster-level multiple comparison corrected p < 0.05. Slices progress from posterior (top-left) to anterior (bottom-right)
Fig. 4.
ROI-average group-mean drug time-course curves during ketamine infusion are plotted for anterior cingulate cortex (ACC), thalamus, orbitofrontal cortex (OFC), and the left caudate nucleus. The percentage change in BOLD signal during ketamine infusion is plotted over time. Error bars indicate standard error of the mean. Risp+Ket = Ketamine activation after risperidone pretreatment.
Table 1
Blood plasma levels for ketamine and risperidone in each of the 4 subjects. Acquisition of blood samples occurred immediately after scanning. Drug levels were determined from heparinized plasma by Tandem Labs using gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Scan 1: Ketamine</th>
<th>Scan 2: Ketamine after Risperidone Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma ketamine (ng/mL)</td>
<td>Plasma ketamine (ng/mL)</td>
</tr>
<tr>
<td>RLc13</td>
<td>114</td>
<td>129</td>
</tr>
<tr>
<td>RRb13</td>
<td>95</td>
<td>89</td>
</tr>
<tr>
<td>RZo13</td>
<td>103</td>
<td>72</td>
</tr>
<tr>
<td>RNe13</td>
<td>106</td>
<td>94</td>
</tr>
</tbody>
</table>
Table 2
Summary of brain areas activated in whole-brain, group-level analysis during ketamine infusion, and ketamine infusion after risperidone pretreatment. Multiple comparison corrected cluster-level p < 0.05

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Brain Areas Activated at corrected p &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>Bilateral: superior frontal gyrus, middle frontal gyrus, supplementary motor area (SMA), anterior cingulate cortex (ACC), cingulate gyrus, insula, superior temporal gyrus (STG), middle temporal cortex, hippocampus, parahippocampal cortex, precuneus, posterior parietal cortex, paracentral lobule (PCL), primary somatosensory cortex (S1), primary motor cortex (M1), thalamus, caudate, putamen, cerebellum and brainstem</td>
</tr>
<tr>
<td>Risperidone + Ketamine</td>
<td>Bilateral: superior frontal gyrus, SMA, M1, parahippocampal cortex, hippocampus, brainstem, cerebellum. Left hemisphere: cingulate gyrus, insula, STG, middle temporal cortex</td>
</tr>
</tbody>
</table>

*Psychopharmacology (Berl)*. Author manuscript; available in PMC 2017 March 01.
Group-level brain activation in *a priori* areas of interest (ROIs) during ketamine infusion, ketamine infusion following pretreatment with risperidone, and the difference between the two conditions. The table reports median t-stat and number of voxels for clusters of significant (multiple comparisons-corrected p<0.05) activation within each ROI. Only ROIs with significant clusters are shown, NS = no significant cluster

<table>
<thead>
<tr>
<th>ROI</th>
<th>Ketamine median t-stat (voxels)</th>
<th>Risperidone+Ketamine median t-stat (voxels)</th>
<th>Risperidone Subtraction median t-stat (voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate cortex</td>
<td>5.14 (148)</td>
<td>NS</td>
<td>4.79 (132)</td>
</tr>
<tr>
<td>Mid-cingulate cortex</td>
<td>5.78 (95)</td>
<td>NS</td>
<td>4.11 (40)</td>
</tr>
<tr>
<td>Left anterior superior temporal gyrus</td>
<td>4.28 (75)</td>
<td>NS</td>
<td>4.37 (53)</td>
</tr>
<tr>
<td>Right anterior superior temporal gyrus</td>
<td>5.01 (78)</td>
<td>4.47 (75)</td>
<td>NS</td>
</tr>
<tr>
<td>Supplementary motor area</td>
<td>4.47 (49)</td>
<td>4.65 (44)</td>
<td>5.24 (30)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>5.27 (151)</td>
<td>4.43 (92)</td>
<td>NS</td>
</tr>
<tr>
<td>Left caudate</td>
<td>3.71 (91)</td>
<td>NS</td>
<td>4.46 (81)</td>
</tr>
<tr>
<td>Right caudate</td>
<td>4.21 (108)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>4.13 (15)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Precuneus</td>
<td>4.46 (231)</td>
<td>5.32 (45)</td>
<td>4.29 (52)</td>
</tr>
<tr>
<td>Putamen</td>
<td>4.07 (219)</td>
<td>4.63 (93)</td>
<td>4.29 (106)</td>
</tr>
</tbody>
</table>
Table 4

Behavioral ethogram indicating clinical ratings of behavior at baseline and following acute risperidone challenge (0.06 mg/kg, i.v.). Mean and standard deviation of behavioral ratings across subjects are presented for baseline and at 15-minute intervals post-injection.

<table>
<thead>
<tr>
<th>Ethogram</th>
<th>Baseline (Mean ± standard deviation)</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>75 min</th>
<th>90 min</th>
<th>105 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye blinking (#/30s)</td>
<td>7.92 ± 2.06</td>
<td>2.08 ± 0.96</td>
<td>0.92 ± 1.07</td>
<td>1.75 ± 0.32</td>
<td>3.67 ± 3.28</td>
<td>2.52 ± 2.32</td>
<td>1.67 ± 0.27</td>
<td>2.5 ± 1.69</td>
<td>2.41 ± 0.99</td>
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<tr>
<td>Tongue protrusions (#/30s)</td>
<td></td>
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<tr>
<td>Oral facial dyskinesias (#/30s)</td>
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<tr>
<td>Chewing (#/30s)</td>
<td>0.08 ± 0.17</td>
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<tr>
<td>Sedation (0–3)</td>
<td>2.75 ± 0.50</td>
<td>2.5 ± 1</td>
<td>2.75 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>2.5 ± 0.58</td>
<td>2.75 ± 0.5</td>
<td>2.5 ± 0.58</td>
<td>2.75 ± 0.5</td>
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<tr>
<td>Stereotypy (0–3)</td>
<td></td>
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<tr>
<td>Dystonia head and neck (0–3)</td>
<td></td>
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<tr>
<td>Dystonia trunk (0–3)</td>
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<tr>
<td>Dystonia upper limbs (0–3)</td>
<td>0.50 ± 1</td>
<td>0.5 ± 1</td>
<td>0.5 ± 1</td>
<td>0.25 ± 0.43</td>
<td>0.25 ± 0.5</td>
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<tr>
<td>Dystonia lower limbs (0–3)</td>
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<tr>
<td>Bradykinesia (0–3)</td>
<td>0.25 ± 0.50</td>
<td>0.25 ± 0.5</td>
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<tr>
<td>Tremor (0–3)</td>
<td>0.25 ± 0.50</td>
<td>0.25 ± 0.5</td>
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<tr>
<td>Salivation (0–3)</td>
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<tr>
<td>Locomotor activity (−3 to 3)</td>
<td>−2.0 ± 2.0</td>
<td>−2.25 ± 1.5</td>
<td>−2 ± 2</td>
<td>−2.25 ± 1.3</td>
<td>−2.75 ± 0.5</td>
<td>−2.5 ± 1</td>
<td>−2.75 ± 0.5</td>
<td>−2.5 ± 1</td>
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</tr>
<tr>
<td>Reactivity (−3 to 3)</td>
<td>−2.5 ± 1.0</td>
<td>−2.25 ± 0.96</td>
<td>−2.25 ± 1.5</td>
<td>−1.75 ± 1.09</td>
<td>−1.5 ± 1.3</td>
<td>−0.75 ± 0.5</td>
<td>−0.75 ± 0.5</td>
<td>−0.75 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>