Investigation of Vertical Translatability of Awake Pharmacological MRI in Non-Human Primate - A Buprenorphine Challenge Study

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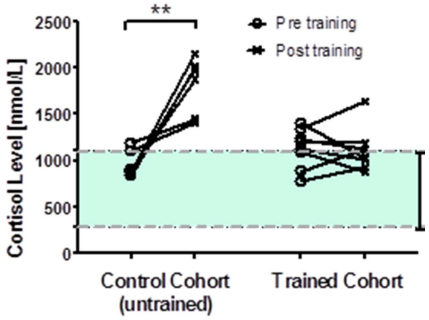
Target Audience: Researchers involved in pre-clinical fMRI model development the CNS effect of drug action.

Purpose: Pharmacological MRI (phMRI) is a neuroimaging technique that allows characterization of changes in neural activities following drug challenge, in which pharmacodynamic (PD) responses can be determined and thereby offers PD biomarkers to delineate underlying biological consequences of drug actions [1, 2]. Notably, in most preclinical imaging studies, animals are anesthetized during data acquisition to minimize movements; however, the use of anesthesia could attenuate basal neuronal activities and undesired anesthetic-drug interactions likely confound drug-induced brain activation patterns [3, 4]. Significant efforts have been made to establish awake imaging in rodents [3] as well as non-human primates (NHP) [4-8]; however comparison and validation of phMRI data as translational endpoints across species remain to be explored. Herein, we established an awake NHP imaging model that encompassed comprehensive acclimation procedures and animal pre-selection processes (behavioral scoring and cortisol measurements) using a dedicated animal restrainer [5]. Using a cerebral blood volume (CBV)-based phMRI approach, we delineated differential responses of brain activities elicited by the systemic administration of buprenorphine (0.03 mg/kg iv) that has been evaluated in human and conscious rats [9, 10]. We hypothesized buprenorphine-induced phMRI maps should parallel μ-opioid receptor distribution in the brain and aimed to investigate translatability of awake NHP findings across rodent and human data.

Materials and Methods: ***Animals and Awake Training:*** Female cynomolgus macaques (4-6 yrs, 2-4 kg, n=12) were studied at Maccine's facility (Maccine Pte Ltd, Singapore) in accordance with IACUC guidelines. Awake training protocol includes *restrainer training* (5 mo.), *mock scanner training* (5 mo.) and *head-post surgery and head-restraint tethering training* (2 wk.) phases. During training sessions, each animal's behavioral gestures (e.g. vocalization, excessive movement, head/body turn) were monitored and scored. ***phMRI:*** The rCBV fMRI data (GE-EPI, TR/TE = 3 s/21 ms, in-plane pixel size=1×1 mm^2, 2 mm slice thickness, 24 slices) were acquired on a 3T Siemens MRI scanner. Well-habituated animals (n=4) were selected for both awake and anesthetized (~1% isoflurane) imaging. Imaging protocol included (i) 5-min baseline (pre-contrast agent), (ii) bolus injection of the contrast agent, Feraheme® (7.5 mg/kg iv) via a tail vein line, (iii) 10-min pre-drug baseline, (iv) infusion of either buprenorphine at 0.03 mg/kg iv or saline vehicle over a 3-min period, and (v) 20-min post-drug data acquisition. High resolution brain anatomical images (TR/TE = 700/13 msec, in-plane pixel size = 0.67×0.67 mm^2) of individual animals were also acquired to co-register with a standard monkey brain atlas [11] for further group-level and ROI analyses. ***Data Analysis:*** All data analyses were conducted using FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and in-house Matlab programs. A GLM was used for the subject-level analysis, where a ramp function was exploited to model the buprenorphine pharmacokinetic response. Motion parameters and mean time-course signals extracted from ventricles and white matter were included as nuisance regressors in unbiased univariate linear regression analyses. Group comparisons were done using a mixed-effects paired *t*-test (FSL FLAME) to determine the group mean of the differential effect of vehicle and buprenorphine in both anesthetized and awake animals.

Results and Discussion: Figure 1 illustrates the cortisol levels measured pre- and post-training, where no significant change observed from trained cohort confirm the effectiveness of training procedures. These results, along with behavioral scores, were used to select better habituated animals to proceed to the next phase of training and eventually the imaging study. Group-level analyses of phMRI data revealed that buprenorphine significantly activated brain regions including, thalamus, striatum, frontal and cingulate cortices (paired *t*-test, versus saline vehicle, $p<0.05$, n=4) in awake NHPs (see Fig. 2), while no significant change was found in these animals imaged under anesthetized condition (data not shown). This observation is strikingly consistent with μ-opioid receptor distribution depicted by [6-O-[^{11}C]methyl]buprenorphine ([^{11}C]BPN) PET study in baboons [12] and post-mortem human brain data [13]. Also, our awake NHP results are consistent with human buprenorphine phMRI maps [9, 10], while differences can be found in comparison to conscious rats data [10], elucidating the need to investigate the vertical translatability of awake phMRI platform.

Conclusion: In summary, our work highlights the utility and importance of awake NHP phMRI for the development of translational imaging biomarker for drug research.



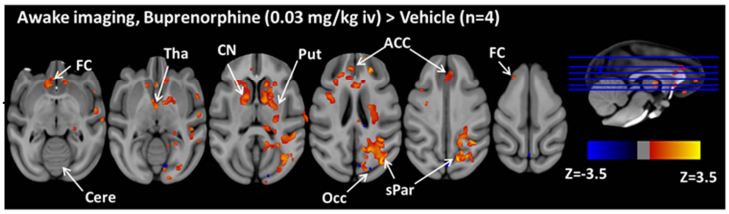


FIG 1 (top) Plasma cortisol concentrations measured from the animals (n=6) at pre- and post-90-minute training session. Cortisol levels are significantly elevated in untrained/control animals, while no significant change was observed in habituated animals (paired t-test, **p<0.01, n=6). The dash lines highlight the range of normal cortisol concentration observed in cynomolgus monkeys (275.9–1103.6 nmol/L). **FIG 2 (bottom)** Brain activation patterns showing significant effect of buprenorphine versus vehicle (paired *t*-test, $p<0.05$, n=4) under awake condition. Buprenorphine activates brain regions with a high density of μ-opioid receptor, including frontal cortex (FC), thalamus (Tha), anterior cingulate cortices (ACC), caudate nucleus (CN), putamen (Put), and superior parietal lobule (sPar), and limited deactivation was found in occipital cortex (Occ), whilst no significant difference was found in anesthetized animals.

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