# Measurement of μ-Opioid Receptor Driven Neurovascular Coupling Signals using Simultaneous PET/MRI

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**Target Audience** Neuroimaging researchers, neuroscientists interested in multimodal imaging, pharmacologists, and addiction researchers.

**Purpose** Opioid drugs are the most effective analgesics for pain management, but opiates as a general class of drugs have significant abuse liability. Although the molecular mechanisms underlying the development of opioid addiction remain incompletely understood, the interaction between the brain's opioid and dopamine systems has been highlighted as potentially pivotal in opioid addiction. Simultaneous PET/MRI is a novel imaging technology and could be used to investigate inter-related receptor system interactions and to dissect complex fMRI signals into neurochemical constituents. In order to develop simultaneous PET/MRI as a tool for investigating the neurobiology of addition and other brain disorders, we aim to measure the relationship between receptor occupancy and fMRI response, and to examine how different receptor systems contribute to a composite fMRI signal. Specifically, we administered pharmacological doses of an opioid antagonist (naloxone) and an agonist (remifentanil) to nonhuman primates (NHPs), and measured changes in cerebral blood volume (CBV) and μ-opioid receptor occupancies using simultaneous PET/MRI.

**Methods** Ten simultaneous and dynamic PET/MRI scans were acquired on two NHPs (male macaques, ~10-12 kg). Animals were anesthetized with isoflurane and mechanically ventilated. Physiological parameters were monitored continuously and maintained within normal ranges. All images were acquired on a 3T Siemens TIM-Trio with a BrainPET insert and a custom PET-compatible 8-channel array coil. PET/MRI scans were acquired from each animal using a μ-opioid selective radiotracers, [$^{11}$C]carfentanil,. Radiotracer (~10 mCi) was given as a bolus-infusion to obtain steady state equilibrium. PET data were stored in list mode and binned into 1-min frames. CBV-fMRI data were obtained following an iron oxide (Feraheme, 10 ug/kg, i.v.)[1,2] injection. Graded doses of an opioid receptor antagonist, naloxone (baseline, 0.01, 0.03, and 0.05 mg/kg) were given intravenously as a challenge at nominally 35 min post radiotracer bolus injection. In addition, one dose of a potent μ-opioid agonist, remifentanil (10 μg/kg) was used as the challenging drug. All data were motion corrected, skull stripped, spatially smoothed and registered to a standard NHP atlas[3]. PET time activity curves (TACs) were analyzed for receptor binding potentials referenced to a non-displaceable compartment ($BP_{ND}$) using the simplified reference tissue model (SRTM)[4]. A gamma-variant function was used to model the PET and fMRI temporal response to drug challenge. The time-to-peak response of the gamma function was adjusted to minimize the $\chi^2$/DOF of the general linear models (GLM) for fMRI and PET data. Changes in fMRI signal intensity were converted to CBV changes using methods described previously[1,2].

**Results** All results were an averaged from the two animals studied. For the baseline condition, PET $BP_{ND}$ maps showed a high-level of specific binding in the thalamus, caudate, putamen, frontal cortex (**Fig 1**). μ-Opioid receptor $BP_{ND}$ and percent CBV reduced in a dose-depended manner to naloxone challenges (**Fig 1**). The largest $BP_{ND}$ reductions were observed in the thalamus and caudate, while the largest CBV changes were observed in the putamen (**Fig 1**). Regional analysis of the $BP_{ND}$ and CBV data **(Fig 2)** revealed a linear coupling relationship. At a given receptor occupancy, the ratio of putamen:caudate %CBV change was ~1.7. A μ-opioid agonist (remifentanil) challenge caused rapid negative CBV change in the caudate/nucleus accumbens (NAc) followed by sustained positive CBV change in the putamen and caudate/NAc **(Fig 3)**.

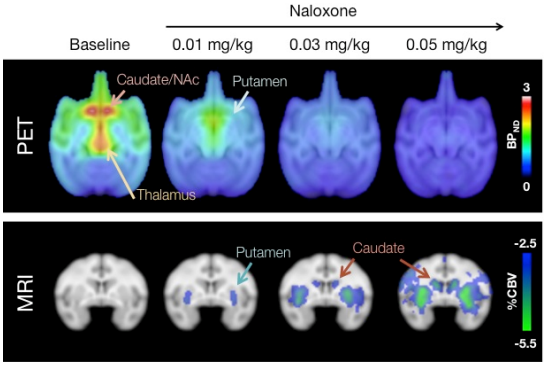


**Fig 1. Simultaneously collected CBV-fMRI and receptor PET with graded-doses of opioid antagonist (naloxone) challenges.** (a) μ-opioid receptor binding potential ($BP_{ND}$) in response to naloxone challenge. (b) GLM results of opioid-antagonist induce CBV changes.

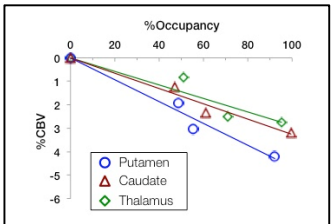


**Fig 2.** Regional %CBV vs. %Occupancy data shows a linear coupling relationship.

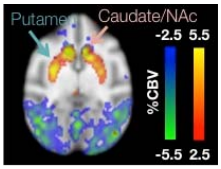


**Fig 3. μ-opioid agonist (remifentanil, 10 μg/kg) evoked fMRI.** A biphasic CBV is shown in the caudate/NAc, while a positive CBV is observed in the putamen.

**Discussion** Baseline μ-opioid receptor binding corresponded well to known distribution of μ-opioid receptors in NHPs. A dose of 0.05 mg/kg naloxone achieved >90% receptor occupancy. Opioid receptors localize primarily on the pre-synaptic terminals and typically exert their effects by modulating other monoamine neurotransmitter systems. Opioid antagonist (naloxone) induced a negative CBV response, which could be due to activating the inhibitory neurotransmitter, GABA, and/or its downstream effects (i.e. GABA depletes basal level of dopamine in the basal ganglia). Based on our regional analysis, the putamen:caudate CBV ratio (~1.7) at a given receptor occupancy matched the known difference of the basal dopamine occupancy[1,2], suggesting the possibility that the CBV responses observed in the basal ganglia are dominated by the indirect opioid-dopamine mechanism. A μ-opioid agonist (remifentanil) challenge evoked robust bi-directional CBV-fMRI responses in the basal ganglia. We have demonstrated in a previous study that the opioid disinhibition mechanism causes endogenous dopamine release in the caudate/NAc[5]. Endogenous dopamine binds preferably to the inhibitory dopamine D2/3 receptors (particularly D3) and may be responsible for the initial negative CBV, while the sustained positive CBV response could potentially be due to the shift of dopamine binding to the excitatory dopamine D1/5 receptors or the disinhibition of GABA. This shift in receptor function, which causes a biphasic CBV signal in the basal ganglia, has been demonstrated in rodents and NHPs[6]. Future pharmacological studies modulating the GABA and dopamine systems are needed to confirm the opioid direct vs. indirect modulations on the fMRI signals.

**Conclusions** Using simultaneous PET/MRI with pharmacological challenges in NHPs, we determined the engagement of the opioid system and the resulting CBV-fMRI implicated a dopaminergic component in limbic basal ganglia. Simultaneous PET/MRI data acquisition provides the unique opportunity to directly relate neurochemical events to functional responses. As such, PET/MRI provides a powerful tool for studying the impact of neurotransmission on brain function, and has great potential to facilitate drug development.

**References:** 1. Mandeville JB, NeuroImage, 2013. 2. Sander CY, et al., PNAS, 2013. 3. McLauren et al., NeuroImage, 2010. 4. Lammertsma, NeuroImage, 1996. 5. Wey et al., ISMRM, 2014. 6. Jenkins, 2013, NeuroImage. ***This work was supported by NIDA K99DA037928.***