# INTERNALIZATION OF DOPAMINE RECEPTORS IMAGED IN VIVO BY SIMULTANEOUS PET/FMRI

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Target Audience: Neuroscientists and multi-modal imaging researchers.

Purpose: Receptor internalization is a homeostatic neuro-adaptation that occurs in vitro in response to large doses of agonist drugs[1]. Non-invasive imaging with fMRI or PET has provided indirect indications that internalization may affect signals: fMRI responses to drugs have reported spatially varying temporal profiles[2], with one explanation being a dynamic change in the concentrations of receptors. PET displacement studies have shown prolonged decreases in specific binding, with one explanation being that internalized receptors keep the signal down[3]. While PET or fMRI alone provide only suggestive evidence about receptor internalization, simultaneous PET/fMRI can yield much better insight into internalization dynamics by a comparison of simultaneous signal changes. For this purpose, graded doses of D2 receptor agonist challenges were applied in a non-human primate (NHP) model during the acquisition of dynamic simultaneous PET/fMRI. We then propose a simplified model that links signal change from PET with fMRI and derive an internalization index from combined PET/fMRI signal changes.

Methods: Imaging data were acquired in two NHPs (rhesus macaque) on a 3T simultaneous PET/MR scanner for a duration of 100 min., using a bolus plus continuous-infusion method with the radiotracer [$^{11}$C]raclopride for dynamic PET and gradient-echo echo-planar-imaging for fMRI. Iron oxide was injected before each scan to improve fMRI detection power. Six studies were carried out with increasing doses of the D2 agonist quinpirole injections (0.1, 0.2, 0.3 mg/kg) at 35 min. after radiotracer injection. fMRI data were analyzed with the GLM and %CBV changes computed according to known methods[4]. PET data were analyzed with a reference tissue model, and a dynamic binding potential ($DBP_{ND}$) was derived as a measure of specific binding[5].

Results: Negative %CBV changes, consistent with the known inhibitory effect of D2-receptor stimulation, were dose-dependent and localized to the basal ganglia. Occupancies of quinpirole increased with dose, with peak occupancies up to 78%. Temporal changes in CBV showed a fast response that peaked within ~2 min. and returned to baseline within ~10 min. (Fig. 1). Contrary to that, PET time-activity-curves showed a prolonged signal decrease in regions of specific binding that stayed down for the duration of the experiment (Fig. 2). Based on both the PET and fMRI time courses, we computed an internalization index that reflects the time course of internalized receptors (Fig. 3).

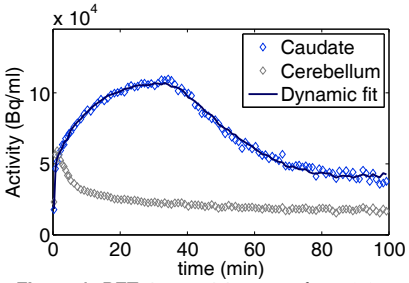


**Figure 1**: PET time activity curve for a 0.3 mg/kg injection of quinpirole.

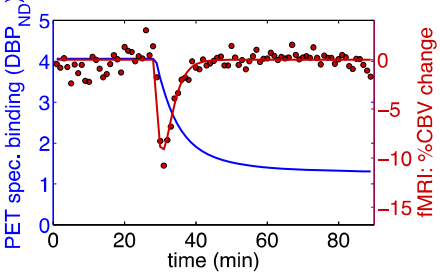


**Figure 2**: Specific receptor binding measure from PET in caudate and %CBV response for a 0.3 mg/kg quinpirole challenge.

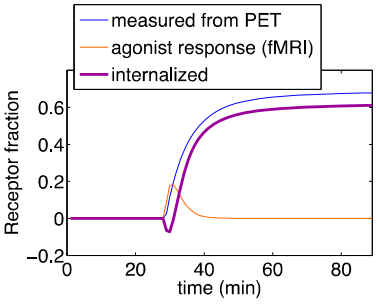


**Figure 3**: Model estimate of internalized receptor fraction due to 0.3 mg/kg quinpirole challenge.

Discussion: While D2 antagonist challenges, which are not generally considered to induce receptor internalization, have shown similar temporal responses compared to PET specific binding[5], we observed divergent temporal responses with D2 agonist challenges. Intuitively, internalization of receptors will abbreviate the fMRI response by down-regulating the targets of agonist, while internalization will prolong the PET response by providing a pool of internalized receptors that can be bound by PET ligand with reduced affinity. Our proposed model assumes that fMRI reflects an instantaneous measurement of changes in occupancy, with dopamine and quinpirole being in dynamic equilibrium: $\Delta CBV(t) \propto \left(1-\theta_{DA}^{(0)}\right)\theta_Q(t)$, where $\theta$ denotes the fraction of basal dopamine (DA) or quinpirole (Q) occupancy. Combining this expression with the law of mass action applied to the postsynaptic receptor population, we can derive an index of receptor internalization that is computed by the difference in scaled quinpirole occupancy and CBV timecourse: $I(t) = \left(1-\theta_{DA}^{(0)}\right)\theta_Q(t) - \alpha\Delta CBV(t)$, where $\alpha$ is a constant derived experimentally. With this model, internalization occurs very quickly with an internalization constant of 10.4 min. and receptors stay internalized beyond the duration of the experiment. These measurements are in concordance with *in vitro* measures that have shown similar internalization constants with quinpirole[1]. While the assumptions in the simplified model involve instantaneous changes, a compartmental model incorporating kinetic parameters and solving for the internalized receptor fraction could yield further insight into the dynamics of receptor adaptation mechanisms.

Conclusion: Mechanisms of receptor internalization have been demonstrated *in vitro*, but to date, there has not been a method for detecting internalization *in vivo*. In this work, we showed for the first time how to derive an *in vivo* index of receptor internalization non-invasively with PET/fMRI, by assessing the temporal divergence of fMRI and PET signals due to an agonist-induced response at D2 receptors. This may lead to further insights into *in vivo* physiological adaptations at targeted receptor systems.

References: [1]Guo N, Guo W, Kralikova M, et al. Impact of D2 Receptor Internalization on Binding Affinity of Neuroimaging Radiotracers. Neuropsychopharmacology 2009;35:806–817. [2]Liu CH, Greve DN, Dai G et al. Remifentanil administration reveals biphasic phMRI temporal responses in rat consistent with dynamic receptor regulation. NeuroImage 2007;34:1042–1053. [3]Skinbjerg M, Liow J-S, Seneca N, et al. D2 dopamine receptor internalization prolongs the decrease of radioligand binding after amphetamine. NeuroImage 2010;50:1402–1407. [4]Mandeville JB, Marota JJA, Kosofsky BE et al. Dynamic functional imaging of relative cerebral blood volume during rat forepaw stimulation. MRM 1998;39:615–624. [5]Sander CY, Hooker JM, Catana C, et al. Neurovascular coupling to D2/D3 dopamine receptor occupancy using simultaneous PET/functional MRI. PNAS 2013.