Pharmacological MRI – *In Vivo* Imaging Platform for Pre-clinical CNS Drug Discovery

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1 BACKGROUND

Pharmacological MRI (phMRI) [1] is a powerful method of functional neuroimaging with unique combination of relevant characteristics for preclinical *in vivo* drug testing in CNS indications, such as high throughput, whole brain coverage, high resolution, versatility of the dosing and measurement paradigms

PhMRI is sensitive to local changes in brain metabolism by detecting fluctuations in blood oxygenation or cerebral blood volume in (de-)activated brain region (Figure 1). Blood oxygenation level dependent (BOLD) contrast is a composite readout of changes in oxygenation and flow of the blood within imaging voxel. Relative cerebral blood volume (rCBV) imaging requires administration of external contrast agent (e.g. microscopic iron particles). Resulting rCBV signal change upon pharmacological stimulation is directly proportional to changes in local contrast agent concentration.

Most commonly, phMRI is used to measure magnitude, time profile and spatial distribution of brain activation after target engagement upon acute test compound delivery during the imaging. Animals may also undergo pretreament with test compound or vehicle before imaging with following relevant pharmacological challenge during the phMRI session.

Modulation of the psychostimulant response in phMRI as a potential functional *in vivo* biomarker of novel drugs' effect was explored in this characterization framework using several combinations (Table 1).

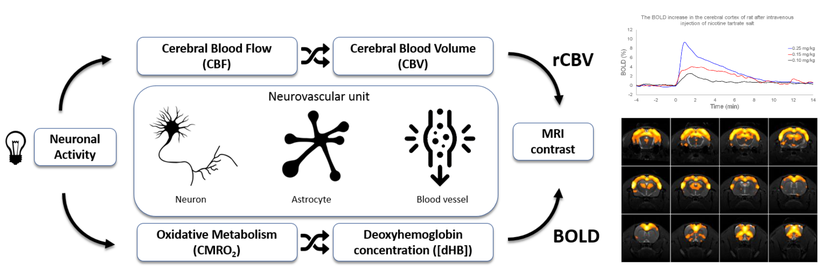

Figure 1. Physiological basis of Pharmacological MRI. Neurovascular unit and coupling of neuronal activation to local hemodynamic response measured by BOLD and rCBV phMRI modalities.

2 MATERIALS AND METHODS

Animals

All experiments were carried out according to the National Institute of Health (NIH) guidelines for the use of laboratory animals, and approved by the National Animal Experiment Board. Male Wistar rats weighing ~320-400 g, were group-housed in a controlled environment with food and water available *ad libitum*.

Animal Preparations and phMRI Procedure

Rats were anesthetized with isoflurane (5% induction, 2% for maintenance) in N_2/O_2 gas 70/30 for insertion of cannulae for blood gases analysis (femoral artery) and administration of experimental compounds (femoral vein or intraperitoneal space). After surgery, Isoflurane anesthesia was adjusted to 1.2-1.5%. Animals were tracheotomized and ventilated, with muscle relaxant (pancuronium bromide 1 mg/kg) administered intravenously while attaching the tracheal tube to mechanical ventilator (Inspira ASVV, Harvard Apparatus, Inc.). Study schematics for rCBV and BOLD protocols are presented in Figure 2.

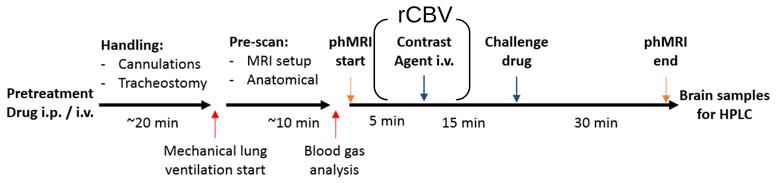

Figure 2. Example of phMRI experiment timeline. Contast injection is used only for rCBV measurements.

Imaging and Data Analysis

Pharmacological MRI acquisitions were performed in a horizontal 7T Bruker MRI system (Bruker Biospin GmbH, Ettlingen, Germany). A 72 mm volume coil was used for transmission and a two-element rat brain surface coil for receiving (both from Rapid Biomedical GmbH, Rimpar, Germany). Rats were fixed to a preheated MRI holder with a bite bar and ear pins for head restraint.

For anatomical reference, T2w TurboRARE images covering the whole brain were acquired in high resolution.

Functional BOLD phMRI data were acquired continuously in one scan for 50 min (15 minutes baseline, infusion start and 35 mins follow-up imaging (Figure 2) using spin-echo echo planar imaging (SE-EPI) sequence with following parameters: 1800 whole-brain volumes with TR 2 s, TE 40 ms. Coronal slice package contained 12 x 1.5 mm thick slices with in-plane resolution of 400 µm x 400 µm (Figure 4).

For rCBV measurements TurboRARE imaging sequence was used (effTR=30s, effTE=60ms, in-plane resolution 250 µm) with USPIO (Ultrasmall Super Paramagnetic Iron Oxide, 20 mg/kg) intravenous contrast agent injected after 5 min of pre-contrast baseline (Figure 2).

Data were preprocessed (motion-corrected, smoothed and coregistered to atlas) using SPM8 /MATLAB. After preprocessing, signal time-series were extracted from 32 regions of interest (ROI) shown in Figure 3:

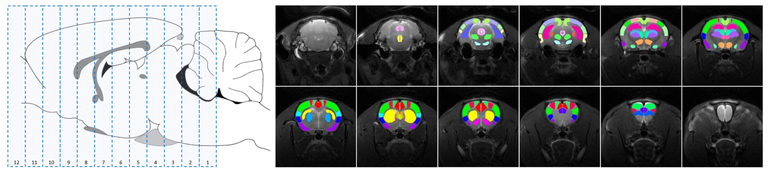

Figure 3. Slice pack placement and regions of interest (ROI) atlas used for phMRI acquisition and data analysis.

To quantify observed responses, area under the curve (AUC) was calculated for 40 time points (20 min) after the compound dosing. Only medial prefrontal cortex (mPFC) shown here as a representative area with highest signal-to noise ratio (SNR).

Very slow drifts in the signal were corrected using inear fitting of the baseline signal. Raw MRI signal was converted into rCBV time series using equation 1:

$$rCBV(t) = \frac{\ln\left(S(t)/B(t)\right)}{\ln\left(B(t)/S_{PRE}\right)} \quad (1)$$

where S(t) – MRI signal after stimulation, B(t) – average post-contrast MRI signal, Spre – average (5 timepoints) pre-contrast baseline MRI signal.

Compounds and experimental groups

In this study we used pharmacological MRI to test agonist-antagonist interactions of common dopaminergic and noradrenergic compaunds, namely intravenously administered amphetamine and yohimbine versus matching D1 dopamine receptor blocker halobenzazepine (SCH23390, Tocris, UK) and α1 receptor antagonist medetomidine (Orion Pharma, Finland). All test compounds were prepared fresh on the day of experiment and were pre-warmed to 36°C temperature before slow bolus injection.

High performance liquid chromatography (Agilent 1260 Infinity II HPLC-system) was used as an auxilary measurement for dopamine and dopamine-derived metabolites level determination in fresh-frozen striatal and cortical samples in SCH23390, amphetamine and control groups (groups 1-3, Table 1).

Animals were randomly allocated into the following treatment groups:

Table 1. Experimental groups with pre-treatment and challenge compounds, doses and imaging methods.

Group	Pre-treatment	Dose and route	Challenge	Dose and route	n	MRI
1	Saline	1 ml/kg i.v.	Saline	1 ml/kg i.v.	4	rCBV
2	Saline	1 ml/kg i.v.	Amphetamine	1 mg/kg i.v.	4	rCBV
3	SCH23390	1 mg/kg, i.p.	Amphetamine	1 mg/kg i.v.	4	rCBV
4	Saline	1 ml/kg i.v.	Yohimbine	1 mg/kg i.v.	3	BOLD
5	Medetomidine	0.1 mg/kg, i.v.	Yohimbine	1 mg/kg i.v.	3	BOLD

3 RESULTS

Both phMRI readouts were sensitive to hemodynamic brain responses to pharmacological stimuli. Amphetamine-induced rCBV signal change peaked at 22 min post dosing at 48% ± 6% (Mean ± SEM). The D1 antagonist SCH23390 caused attenuated amphetamine response by 36% (Figure 4). In saline/yohimbine-treated rats negative BOLD response peaked at 63 sec at -3.4 ± 0.23% with medetomidine attenuation of that effect by 31% (Mean ± SEM, Figure 5).

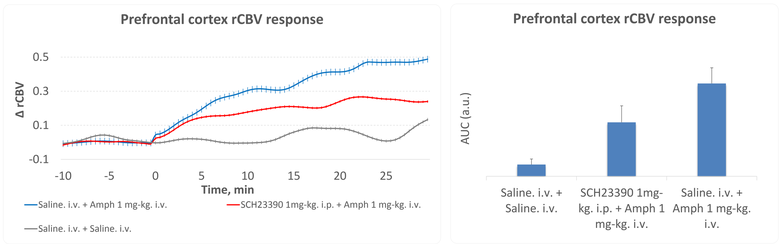

Figure 4. Left: rCBV phMRI signal time series from rat medial prefrontal cortex treated with amphetamine and its effect reversal with D1 receptor antagonist SCH23390. **Right:** Quantification of the 20 min area under the curve (AUC), Mean ± SEM.

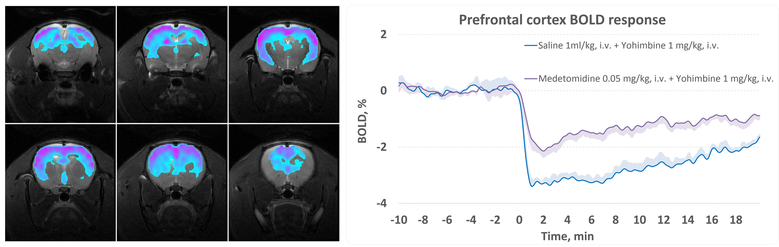

Figure 5. Left: BOLD phMRI statistical activation map (T-score, FDR corrected, p<0.05) of 1 mg/kg yohimbine dosing. **Rigth:** BOLD signal time series from rat medial prefrontal cortex treated with yohimbine and its attenuation with medetomidine (Mean ± SEM).

The HPLC analysis of catecholamines concentrations in fresh-frozen cortical samples demonstrated marked increase in dopamine and Homovanillic acid (HVA) in both saline/SCH23390 and SCH23390/amphetamine groups compared to saline/saline and saline/amphetamine controls. Interestingly combination of SCH23390 and amphetamine caused a pronounced increase in norepinephrine (NE) levels

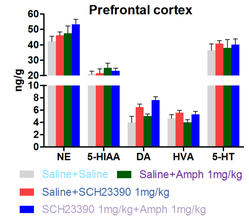

Figure 6 (Right). HPLC catecholamine content analysis from cortical samples in amphetamine and SCH23390 phMRI treatment groups (Mean ± SEM).

4 CONCLUSION

Pharmacological MRI (phMRI) provides a powerful, translatable tool to evaluate neuronal activation patterns following pharmacological challenge [1]. The technique allows spatial and temporal analysis of the responses in brain regions of interest and also provides evaluation of the magnitude of this activation in dose-dependent manner. Modulation of responses of known stimulants by tool compounds can serve as a reference model for novel drugs testing.

In this work, we have used generic psychoactive compounds that target dopamine- and serotoninergic neurotransmitter systems to establish the psychiatry-oriented, optimized platform for phMRI experiment in rats. This experimental design can be applied widely to examine BOLD and rCBV responses of novel CNS acting pharmacological entities in the whole brain, and benchmarking them against known classes of compounds. Finally, this work sets a ground for phMRI experiments in the context of various CNS disease models, to obtain temporal and spatial profile of the studied compounds to advance the early stages of drug discovery.

[1] Leslie RA, James MF. Pharmacological magnetic resonance imaging: a new application for functional MRI. Trends Pharmacol Sci. 2000;21(8):314-8.