BOLD phMRI in the rat on a clinical 3T scanner using cocaine challenge

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Introduction

Driven by an increasing productivity gap, the pharmaceutical industry is looking for better methodologies for compound development. These should help to select only those candidates with the highest chance of successfully demonstrating efficacy in larger-scale clinical trials. Pharmacological MRI (phMRI) [1] is one such methodology which can be used to study the effect of a drug on brain activation in both the preclinical and clinical situation. Most preclinical phMRI experiments are performed on specialized preclinical MRI scanners [2], which are less readily available and increase complexity for translation to clinical studies. The aim of this study was to test the feasibility of performing a cocaine BOLD phMRI study in the rat brain using a clinical 3T MRI scanner.

Methods and Materials

Preparation: Sprague-Dawley rats (male, average weight=313±11 g, n=4). Each rat was scanned twice, with scanning sessions performed 1 week apart. In the first scanning session, rats were injected with saline during the scan, in the second session with cocaine (5 mg/kg, i.v.). For each scan the rats were anaesthetized using isoflurane (2.5%) and placed in a dedicated rat support unit (Minerve, Esternay, France). The body temperature was monitored with an optical temperature sensor (Neoptix, Québec City, Canada) and controlled by a heated air flow through the support unit. The depth of the anesthesia was monitored from the respiration rate (ECG trigger unit, Rapid Biomedical, Rimpar, Germany). After respiration rate stabilization at 60-75 breaths/min the isoflurane concentration was decreased to 1.5% and the BOLD phMRI sequence started. At the end of the scanning protocol, a blood sample extracted from the left ventricle was collected and the pH, pCO_2, pO_2 and HCO_3 measured using an i-Stat blood analyzer (Abbott, Birmingham, UK).

MRI: After the animal was positioned in the support unit a 4-channel SENSE rat head coil (Rapid Biomedical) was placed on the skull of the animal and during the preparation phase an optimized SENSE reference scan was acquired. The scan protocol was started by acquiring a high quality anatomical scan via SE sequence with the following parameters: TR/TE: 1745/50ms; Matrix: 256x256; FOV: 4 cm; Resolution: 156x156 µm; slice thickness: 1 mm; Number of slices: 24 from olfactory bulb towards cerebellum; SENSE factor 2; acquisition time: 10 min. When the respiration rate of the animal was stable a SE-EPI BOLD phMRI sequence was started: TR/TE: 1749/60 ms; EPI factor 9; Matrix: 128x128; FOV: 4 cm; Resolution: 312x312 µm; slice thickness: 2 mm; Number of slices: 12; SENSE factor 2; Fat suppression: SPIR; Dynamic scan number: 450; Total acquisition time: 1.5 hours with saline or cocaine injection after 30 min; Temporal resolution: 12 sec.

Data analysis: First the individual subjects were spatially normalized to a stereotaxic rat brain MRI template set [2]. BOLD phMRI time series were analyzed using the pseudo-block method with a temporal resolution of 5 minutes [3]. Volumes of interest (VOI) were delineated from the data using a 3D digital reconstruction of the Paxinos and Watson (1998) rat brain atlas, co-registered with the rat brain template [2]. Statistical Parametric Mapping (SPM5) software (Wellcome Trust Centre for Neuroimaging, London, UK) was used to perform the statistical analysis.

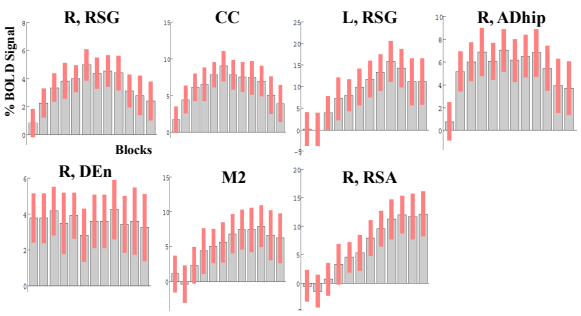

Figure 1: Histograms describe the extracted time series from the peak-responding voxel in a cluster from the areas of interest. Each bar corresponds to 5 min time bins with error bars representing 90% confidence intervals of the BOLD signal change. Abbreviations are explained in the text.

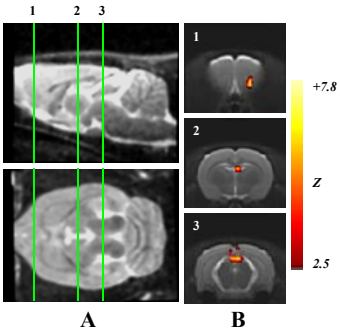

Figure 2: Distribution of significant activations from cocaine vs. placebo subtraction across the time series. (A) position reference of the three slices shown in column B. (B) Map of the focal activations in the 1) piriform cortex, 2) ADHip, 3) retrosplenial cortex. Abbreviations are explained in the text.

Table 1: Statistical values of the peak voxel and the areas comprised by the clusters.

Statistical Values			Anatomical
Cluster Size	*T*- value	*p*-value	Localization
105	7.78	0.000	R, RSG
	7.32	0.000	CC
	5.73	0.001	L, RSG
33	6.36	0.000	R, ADHip
19	6.20	0.000	R, DEn
20	5.96	0.001	M2
10	5.62	0.002	R, RSA

Results

Before this study several MRI sequences were tested *in vivo* by injecting iron-oxide particles (Endorem, Guerbet, The Netherlands) as a model for T2* weighting. The SE-EPI with TE=60 ms was the most preferred sequence based on image quality and T2* sensitivity. Figure 1 shows the bold signal change of these pseudo-blocks compared to baseline signal intensities of the most interesting brain structures: retrosplenial granular cortex (RSG); corpus collosum (CC); antero-dorsal hippocampus (ADhip); dorsal endopiriform nucleus (DEn); secondary motor cortex (M2); retrosplenial agranular cortex (RSA). The letters R and L signify the right side and the left side of the animal, respectively. Significant activation of three different brain structures are visualized in Figure 2 determined via statistical analysis of the average effect over time across groups. Table 1 describes the statistical values of the peak voxel and the areas comprised by the clusters. *T*-statistic maps for the cocaine vs. placebo subtraction have been family wise error (FWE) corrected at $p<0.05$. The reversed contrast placebo vs. cocaine did not yield any significant deactivations following FWE correction. The average blood values were: pH=7.448±0.024; PCO_2=39.7±3.3 mmHg; PO_2=59.9±22.5 mmHg and HCO_3=27.4±1.1 mmol/l (mean±SD). Only the PO_2 blood value differed from the reference blood value of awake rats (90±5.5 mmHg) [4].

Discussion and conclusion

In this study we have demonstrated that phMRI in the rat is possible on a 3T clinical MRI scanner. The significant activations observed in response to cocaine challenge indicate a complex relationship between the BOLD signal changes and the underlying dopamine system [5]. Issues are the use of isoflurane, which suppress brain activation, and the offline blood gas analysis. Structure wise analysis of the BOLD signal did not reveal global variations indicating that variations in PCO_2 did not confound local results. We conclude that preclinical phMRI experiments can be performed using clinical protocols and equipment.

References: 1) Schwarz A, *et al.*, SYNAPSE 54:1–10 (2004); **2)** Schwarz A, *et al.*, NeuroImage 32(2): 538-550 (2006); **3)** McKie, *et al* ., Psychopharmacology (Berl) 180(4): 680-6 (2005); **4)** Brun-Pascaud M, *et al.*, Respir Physiol. Apr;48(1):45-57 (1982); **5)** Chen, *et al.* Magn Reson Med 38(3): p. 389-98 (1997).