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Assessment of rodent brain activity using combined [^{15}O]H_2O-PET and BOLD-fMRI

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ABSTRACT

The study of brain activation in small animals is of high interest for neurological research. In this study, we proposed a protocol to monitor brain activation in rats following whisker stimulation using the short half-life PET tracer [^{15}O]H_2O as a marker for cerebral blood flow. This technique enables the study of baseline and activation conditions in fast succession within the same scanning session. Furthermore, we compared the results obtained from PET imaging with additional BOLD-fMRI data acquired in the same animals within the same anesthetic session in immediate succession. Although the maximum relative signal changes during brain activity observed with PET were substantially higher compared to the BOLD-fMRI results, statistical analyses showed that the number of activated voxels in PET was lower compared to the fMRI measurements. Furthermore, there was a difference in the activation centers in both the shape and location between PET and fMRI. The discrepancy in the number of activated voxels could be attributed to a lower overall contrast-to-noise ratio of the PET images compared to BOLD-fMRI, whereas the difference in the spatial location indicates a more fundamental process, involving the different physiological origins of the PET and BOLD-fMRI response. This study clearly demonstrates that [^{15}O]H_2O-PET activation studies may be performed in small laboratory animals, and shows the complementary nature of studying brain activation using [^{15}O]H_2O-PET and fMRI.

Introduction

The study of the functional processes of the brain is of high interest for basic research and for clinical diagnosis. Positron emission tomography (PET) (Frackowiak and Friston, 1994) using [^{15}O]H_2O (half-life time $T_{1/2}$ = 122 s) as a marker for cerebral blood flow (*CBF*) to map brain activation has been widely employed to investigate brain function in humans (Fox and Mintun, 1989; Fox and Raichle, 1986; Hummel et al., 2009; Payoux et al., 2010) because it allows a rapid alteration between baseline and activation scans due to the short half-life of [^{15}O]. The blood oxygen level dependent effect (BOLD), which has been used more recently in functional magnetic resonance imaging (fMRI) studies of brain activation, reflects the complex interplay between changes in *CBF*, cerebral blood volume (*CBV*), cerebral metabolic rate of oxygen consumption ($CMRO_2$) and oxygen extraction fraction (*OEF*) (Buxton, 2010). Despite the widespread utilization of the BOLD effect to measure brain activation in humans (Brown et al., 2011; Iannetti and Wise, 2007; Luijten et al., 2011) and in animals (Just et al., 2010; Sanganahalli et al., 2008; Seehafer et al., 2010), its physiological bases are not entirely understood (Buxton, 2010; Logothetis et al., 2001; Shulman et al., 2007). Thus, it would be of the utmost interest to measure and compare brain activation using both [^{15}O]H_2O-PET and BOLD-fMRI to cross-validate these two markers of brain function and to deconvolute the complex nature of the BOLD signal.

Brain activation studies in rodents undergoing sensory stimulation and assessed using PET in combination with the tracer [^{15}O]H_2O have, to the best of our knowledge, not been previously described. There have been a few *CBF* measurements performed in rats using PET, and most examine global values (Ose et al., 2012; Watabe et al., 2013; Yee et al., 2005). Previous studies comparing PET and BOLD-fMRI have only been performed in humans and have produced contradictory results. Ramsey and colleagues compared [^{15}O]H_2O-PET with BOLD-fMRI in sequential measurements, where they identified a high correlation in terms of sensitivity between the two methods (Ramsey et al., 1996). Kinahan et al. found a significant mismatch in spatial location between the PET and fMRI activation centers in humans (Kinahan and Noll, 1999). In addition, Joliot et al. also found a mismatch between

Abbreviations: [^{15}O]H_2O, water labeled with the radioactive isotope [^{15}O], used as a PET tracer; AIF, arterial input function; BOLD, blood oxygen level dependent effect; *CBF*, cerebral blood flow; CBV, cerebral blood volume; CG, cingulate cortex; cl, contralateral; $CMRO_2$, cerebral metabolic rate of oxygen consumption; CNR, contrast to noise ratio; EPI, echo planar imaging; fMRI, functional magnetic resonance imaging; FWE, family-wise error correction; il, ipsilateral; OEF, oxygen extraction fraction; PET, positron emission tomography; ROI, region of interest; TE, echo time; TR, repetition time; S1BF, barrel field cortex; S2, secondary somatosensory cortex; V2L, visual cortex; VPM, ventral posterolateral nucleus; *X*, *Y*, *Z*, coordinates in the Paxinos space with bregma = Z = 0.

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the PET and fMRI activation maps in humans (Joliot et al., 1999), whereas a subsequent experiment by Devlin et al. concluded similar, but not identical, PET and fMRI results (Devlin et al., 2000).

To shed additional light on to the relationship between PET and fMRI activation studies, we used a small animal model, which can be extensively examined under more controlled conditions and multiple repetitions. The aim of this study was to compare [^{15}O]H_2O-PET and BOLD-fMRI measurements in rats that were acquired in immediate succession during the same anesthetic session using a whisker stimulus. To achieve this goal, we established a noninvasive *in vivo* brain activation mapping protocol in small animals using [^{15}O]H_2O-PET.

Methods

Animal preparation

All of the animal experiments were approved by the local authorities (Regierungspraesidium Tuebingen). Eight male Lewis rats at an age of 17 ± 4 weeks and a weight 358 ± 16 g (Charles River Laboratories, Sulzfeld, Germany) were used in the combined PET and MR brain activation measurements. Each animal was initially anesthetized with a mixture of 1.5% isoflurane vaporized in air at a gas flow rate of 1.0 L/min. The animals were placed head first in a prone position on a multimodality imaging bed (Bruker BioSpin MRI, Ettlingen, Germany), which is suitable for both PET and MR measurements. Body temperature and respiration rate were monitored and maintained. The animals underwent sequential PET and MR scans during whisker stimulation in immediate succession. The animal position and location of the stimulation electrodes were not altered between the scans. Details can be found in the Supplementary material (S 2.1).

Stimulation

Electrical stimulation of the left whisker pad of the animals was performed using subcutaneously placed needle electrodes. The stimulus frequency (3 Hz), current (3 mA, 500 μs pulse width) and location of the electrodes were not altered between PET and fMRI measurements. However, to ensure an optimal signal-to-noise ratio (SNR) during each measurement, the stimulus paradigm was adapted. Using this adaption on the respective imaging method, conditions such as tracer half-life ($T_{1/2}$ = 122 s) and fast repetitive measurements using fMRI were considered. Thus, for the PET measurement, the stimulation paradigm was applied during the entire activation-scanning period, and switched off during the entire baseline-scanning period. For the fMRI measurement, the 60-s off-periods (baseline) were altered with 30-s on-periods (activation). For additional details, please refer to the Supplementary material (S 2.2).

PET measurements

All the PET measurements used a dedicated small animal PET scanner (Inveon, Siemens Molecular Imaging, Knoxville, USA). A total of 8 [^{15}O]H_2O-PET scans were performed per animal for each imaging session. In two animals deviations from the intended protocol occurred: In one rat only 7 PET scans (instead of 8) were obtained due to problems with the [^{15}O]H_2O delivery. In one animal five activation and three baseline scans were performed due to timing problems with the stimulus generator. The PET images were reconstructed using a 2D filtered backprojection (FBP) algorithm with a matrix size of 256 × 256 × 159, a zoom factor of 2 resulting in a reconstructed voxel size of 0.19 × 0.19 × 0.8 mm^3 and were attenuation and decay corrected.

For additional details, please refer to the Supplementary material (S 2.3).

MRI measurements

A 7 T small animal MRI scanner (ClinScan, Bruker Ettlingen, Germany) was used for the experiments. A 2 × 2 channel rat brain coil was employed for the MR data acquisition. A 3D anatomical turbo spin echo MR scan was performed to enable coregistration of the PET and MR image. After this initial scan, the fMRI-BOLD imaging session began using a gradient echo-based echo planar imaging (EPI) method, which acquired 225 image volumes, 5 baseline blocks (each 60 s), and 5 activation blocks (each 30 s). The EPI sequence parameters were TR = 2000 ms, TE = 18 ms, matrix size = 64 × 64 × 8, voxel size = 0.5 × 0.5 × 1 mm^3, EPI factor of 64, 2 preparation scans, 225 volumes, 5 baseline blocks, with 60 s each, and 5 activation blocks with 30 s each, 7.5 min acquisition time. For additional details, please refer to the Supplementary material (S 2.4).

Data processing: Statistical processing and analysis

The PET and MR statistical analysis of the functional images were performed at a group level using SPM 8 (Welcome Trust Center for Neuroimaging, London, Great Britain). Details on the image preprocessing as well as statistical processing for the PET and fMRI can be found in the Supplementary material (S 2.5–S 2.8, Supplementary Figs. 1–3).

To compare the PET and MR data, the two data sets were resliced during image processing to a resolution of 0.22 × 0.22 × 0.22 mm^3 and smoothed with a Gaussian kernel to a resolution of 1.5 × 1.5 × 1.5 mm^3. The mentioned resampling of the PET and MR data with the same nominal spatial resolution in the compared images clearly supports comparability. However, it should be mentioned, that the resampling to a common resolution using two different, modality-dependent base resolutions may have small effects on the indicated activated areas and SNR values that cannot be completely be ruled out.

Brain activation maps between PET and MR were compared regarding the statistical significance of the activated voxels, number of activated voxels, location of the activation maxima in the T-maps, center of gravity (coordinates in cluster weighted by the respective T-value) of the activated areas, and center position (center of coordinates in cluster without weighting by their T-value).

For the relative blood flow and BOLD signal changes between the activation and baseline, a ROI analysis was performed. The ROIs centered at the T-value maximum of the activation in the il and cl S1BF regions, were drawn using SPM 8 and the MarsBaR ROI Toolbox (MarsBaR Version 0.43) (Brett et al., 2002), based on the summed PET images, which are proportional to the *CBF* (Supplementary material S 2.5, S 2.7). The ROIs had a spherical size of 3 mm centered around the respective PET and fMRI T-value activation maxima or were based on the activated cluster for T-values > 3. The significance was assessed on a $P < 0.05$ level with Student's t-test of all of the activation values versus all of the baseline values. The average differences in the cerebral blood flow (ΔCBF) between baseline and activation were computed using the formula: $\Delta CBF = (CBF_{active} - CBF_{base}) / CBF_{base}$.

Data processing: Comparison of the activated volumes and localization of the activation centers between the PET and fMRI

The differences between the respective MR and PET coordinates were calculated using the norm $\left(\left|\vec{d}\right|\right)$ of the difference vector $\left(\vec{d}\right)$, which gives the Euclidean distance (d) between the two activation foci.

$$d = \left|\vec{d}\right| = \sqrt{(a_1-b_1)^2 + (a_2-b_2)^2 + (a_3-b_3)^2},$$

where (a_1, a_2, a_3) and (b_1, b_2, b_3) are the (X, Y, Z) coordinates of the respective vectors $\vec{a}$ and $\vec{b}$ from a coordinate origin (chosen as the origin of the Paxinos space with $X = 0$, $Y = 0$ and $Z = 0$) to the peak of the PET activation and to the peak of the MR activation in the