Statistical parametric maps of ^{18}F-FDG PET and 3D autoradiography in the rat brain: a cross-validation study.

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KEYWORDS

ABSTRACT

Although specific PET scanners have been developed for small animals, spatial resolution remains one of the most critical technical limitations, particularly in the evaluation of the rodent brain. The purpose of the present study was to examine the reliability of voxel-based statistical analysis (SPM) applied to ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET images of the rat brain, acquired on a small animal PET not specifically designed for rodents. The gold standard for the validation of the PET results was the autoradiography of the same animals acquired under the same physiologic conditions, reconstructed as a 3D volume and analyzed using SPM. **Methods:** Eleven rats were studied under two different conditions: conscious or under inhalatory anesthesia during ^{18}F-FDG uptake. All animals were studied *in vivo* under both conditions in a dedicated small animal Philips MOSAIC PET scanner and magnetic resonance images were obtained for subsequent spatial processing. Then, rats were randomly assigned to a conscious or anesthetized group for post-mortem autoradiography and slices from each animal were aligned and stacked to create a 3D autoradiographic volume. Finally, differences in ^{18}F-FDG uptake between conscious and anesthetized states were assessed from PET and autoradiography data by SPM analysis and results were compared. **Results:** SPM results of PET and 3D autoradiography are in good agreement and led to the detection of consistent cortical differences between the conscious and anesthetized groups, particularly in the bilateral somatosensory cortices. However, SPM analysis of 3D autoradiography also highlighted differences in the thalamus that were not detected with PET. **Conclusion:** This study demonstrates that any difference detected with SPM analysis of MOSAIC PET

images of rat brain is detected also by the gold standard autoradiographic technique, confirming that this methodology provides reliable results, although partial volume effects might make it difficult to detect slight differences in small regions.

1. INTRODUCTION

Positron emission tomography (PET) for *in vivo* imaging of metabolic processes is a powerful tool to study the etiopathogenesis and progression of neurological diseases and to test the efficacy of new therapeutic agents [1]. Recently, specific PET scanners have been developed for small animals which provide smaller fields of view and higher spatial resolution than clinical tomographs. This allows longitudinal functional neuroimaging studies to be undertaken in animal models of various neurological conditions, including Parkinson's disease [2, 3], Alzheimer's disease [4], and epilepsy [5]. Moreover, due to the fact that the same imaging technique can be used for both animal and human studies, methodologies and results obtained from animal models may be easily translated to the clinical setting.

In PET studies of the human brain, voxel-based analysis is the most extensively used methodology for quantification. Statistical parametric mapping (SPM) is the most popular software for this purpose [6]. This software applies a general linear model to each voxel to perform statistical comparisons between images of different subjects. The result is a statistical image that can be used to spatially localize significant information. It should be noticed that use of SPM requires that images from different individuals be spatially normalized, that is, mapped into a common stereotaxic space by using a template image as reference. In comparison to traditional analysis using regions of interest (ROIs),

SPM is faster and includes the whole brain in the analysis, without any preconception about the structures involved in the investigated pathology. Recently, SPM analysis has also been applied to the study of animal models of different neurological diseases [7, 8] and various templates have been developed for spatial normalization of rodent brains [9, 10].

Although the resolution of small animal-dedicated tomographs has significantly improved, high-resolution autoradiography still represents a key reference tool for functional neuroimaging in small animal research. Autoradiography is a widely- available low-cost technique with a spatial resolution approximately one order of magnitude better than even the best small animal PET devices (100-200 micrometers *vs* 1-2 millimeters respectively) [11]. However, autoradiography requires the sacrifice of the animal, thus negating its use in longitudinal studies. These differences between *in vivo* small animal PET and autoradiography are widely known, and several studies have compared the results of these two techniques in detail [12-16]. Autoradiography is inherently a two-dimensional (2D) sampling method in which slices are obtained independently without correspondence between neighboring slices. Therefore, quantification is classically obtained by the labor-intensive procedure of manually drawing ROIs plane by plane. In order to avoid this drawback, several authors have described different methodologies for voxel-based analysis, all of them based on the creation of a three-dimensional (3D) volume stack of aligned autoradiographic slices [17-19]. Moreover, these methodologies have been successfully applied in the study of different animal models of neurological pathologies [20, 21].

In this study, the primary objective was to validate statistical voxel-based analysis of PET rat brain studies performed in our Philips MOSAIC small animal PET tomograph, using high-resolution 3D autoradiography as a reference. To this end, an automated procedure for voxel-based analysis of PET and autoradiography was designed and applied for comparison of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) metabolic studies of the rat brain. For comparative purposes, animals were studied under two different conditions during tracer uptake: conscious and under inhalatory anesthesia (isoflurane), protocols that have been demonstrated to result in clear differences [22]. To the best of our knowledge this is the first study to compare SPM results between 3D autoradiographic volumes and PET images of the rat brain.

2. MATERIALS AND METHODS

2.1. Animals:

Eleven male healthy Sprague–Dawley rats (Harlan Ibérica, Spain) weighing 253 ±14.5 g were individually housed under light-controlled (12:12 light/dark cycle, with lights on at 08:00 h) and temperature-controlled (22 ± 1 ºC) conditions. Animals had *ad libitum* access to food and tap water. Rats were deprived of food for 8 h before both PET and autoradiography studies. The research protocol was approved by the local Animal Ethics Committee (Universidad de Navarra Institutional Committee on Care and Use of Laboratory Animals) and was designed according to European Ethics Committee guidelines (decree

86/609/EEC). All animals were studied with PET, magnetic resonance imaging (MRI) and autoradiography techniques.

2.2. PET Studies

^{18}F-FDG PET imaging was performed at Clínica Universidad de Navarra in a dedicated small animal Philips MOSAIC tomograph (Cleveland, Ohio, USA). This system is based on 14456 GSO crystals with dimensions of 2 x 2 x 10 mm, arranged in 52 rings of 278 crystals each [23]. The crystals are read out by an hexagonal array of 288 photomultiplier tubes. This gantry design leads to a transverse field of view (FOV) 12.8 cm in diameter and with an axial extent of 12.0 cm, designed to image rodents or larger animals such as primates [24]. The scanner operates exclusively in 3D mode. A detailed description of this system can be found elsewhere [25]. The radial spatial resolution of this system is 2.7 mm full width at half-maximum (FWHM) in the radial direction and 3.4 mm FWHM in the axial direction at the center of the FOV [23].

PET studies were conducted for two conditions in all rats (n=11): conscious or under inhalatory anesthesia (2% isoflurane in 100% O_2 gas) during the ^{18}F-FDG uptake period. For each animal, PET in conscious state was performed first and all the studies were completed in a one week interval. In both conditions, animals were anesthetized using isoflurane at the moment of the tracer injection and during the PET studies. Thirty minutes after radiotracer administration via the tail vein (20.2 ± 0.3 MBq in 0.2 ml of saline solution), animals were placed prone on the PET scanner bed with their head positioned in the center of the FOV to perform a static acquisition of 15 minutes. Images were subsequently

reconstructed using an iterative 3D maximum likelihood algorithm (3D Ramla), with 2 iterations and a relaxation parameter of 0.024. Corrections for dead time, decay, random coincidences and scattering were applied. Images were reconstructed on a 128 x 128 x 120 matrix, where the voxel size equals 1 x 1 x 1 mm. PET images were normalized for the injected dose of ^{18}F-FDG and the animal's body weight, resulting in parametric images representing SUV (Standard Uptake Value).

2.3. MRI Acquisition

MRI studies of the same population of rats were performed at Complutense University of Madrid (UCM) fifteen days after PET images. MRI scans were acquired in a BIOSPEC BMT 47/40 (Bruker, Ettlingen, Germany) scanner operating at 4.7 Teslas, and equipped with a 12 cm actively shielded gradient system.

Rats were anesthetized with the same mixture of oxygen and isoflurane, after which they were placed in a prone position inside a 7 cm birdcage radiofrequency probehead with their head maintained in a fixed position. A respiration sensor was used to control the animals during the scanning procedure. Global shimming was performed, followed by three fast spin-echo scout images in the axial, sagittal and coronal directions to localize the brain. The acquisition parameters for these acquisitions were: Repetition Time (TR) = 2100 ms, effective Echo Time (TE) = 60 ms, FOV = 3.6 x 3.6 cm^2, and matrix size = 256 x 128. The acquired data were zero-filled to obtain images of 256 x 256 pixels. The acquisition time for each experiment was 33 s. A 3D fast spin-

echo study was then performed using the following acquisition parameters: TR = 1600 ms, effective TE = 80 ms, FOV = 3.5 x 3.5 x 3.5 cm^3, and matrix size = 128 x 128 x 64. The reconstructed matrix size was 128 x 128 x 128 and the pixel size was 0.27 x 0.27 x 0.27. The total acquisition time for this experiment was 14 minutes.

2.4.   Autoradiography

The regional glucose metabolism of the same animals was evaluated *ex vivo* by autoradiography at the Brain Mapping Unit of Complutense University of Madrid. Autoradiographic measurements were performed after MRI and were completed in a one week interval. ^{18}F-FDG was injected via the tail vein (54.5 $\pm$ 2.9 MBq in 0.2 ml of saline solution). The animals were then divided into two groups based on whether they were conscious (n=5) or under isoflurane anesthesia (2% in 100% O_2; n=6) during the 30 min tracer uptake period. At the end of the uptake period the animals were sacrificed and their brains were quickly and carefully removed and frozen by immersion in cold isopentane in dry ice. Serial coronal brain sections (40 μm thickness) from the olfactory bulb to the level of the cerebellum were cut at -20ºC using a cryostat (Leica CM1850, Germany). One out of every five sections was collected, yielding approximately 100 sections per animal, at a separation of 200 μm. The slices were thaw-mounted onto Superfrost Plus® microscope slides (Menzel-Gläser, Braunschweig, Germany), rapidly heat-dried, and exposed to an autoradiographic film (Agfa Curix RP2 Plus, Mertsel, Belgium) for one hour. The films were then manually developed and left to dry in a warm air stream. All the

autoradiographically labeled brain slices were digitally captured at a resolution of 1300 x 1030 pixels (Leica DC300F, Germany).

2.5. PET analysis

For whole brain analysis, images from the different rats were spatially normalized into a standard stereotaxic space using the Paxinos and Watson [26] coordinate system (Figure 1). Although a T2-weighted MRI template has previously been published [10], neither PET nor autoradiography templates were available. Hence, the normalization procedure required an initial step registering each image to the corresponding MRI scan in order to use the MRI rat template.

Taking into account the spatial resolution of our PET images, automatic registration between the PET and MRI scans was not feasible. Therefore, a manual registration process employing PMOD software (version 3.0; PMOD Technologies Ltd., Adliswil, Switzerland) was used, based on MRI including the skull and scalp and using anatomical structures such as the Harderian glands as reference points. Each MRI scan was normalized to Paxinos space (SPM, Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) and the transformation matrix was applied to each PET image and saved for future application. Prior to statistical analysis, a brain mask obtained from each MRI procedure was applied over the PET scan to exclude extra-cerebral areas. Each of the scans was also individually smoothed with a Gaussian kernel to reduce the impact of misregistration into template space and to improve the signal to noise ratio. The smoothing was performed with a 0.6 mm FWHM filter.

Differences in ^{18}F-FDG uptake between the conscious and anesthetized state were assessed using SPM8 software, including the 11 animals in the two acquisition conditions. A two-sample paired t-test was used to account for the fact that the both conditions were obtained from the same animal. To ensure that the analysis only included voxels mapping cerebral tissue, a default threshold of 80% of the mean uptake inside the brain was selected. Global uptake differences between brain scans were adjusted using the "proportional scaling" SPM option.

A significance level threshold of 0.005 (uncorrected for multiple comparisons) and a minimum cluster size of 200 voxels were selected. The size of the clusters exceeding the threshold and their corrected significance were evaluated and anatomically located using a ROI template [27] in Paxinos stereotaxic space.

2.6. Creation of a 3D Volume of Autoradiography and SPM analysis

The autoradiographic brain images were pre-processed with ImageJ v 1.37 software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). First, a rectangular bounding box (700 x 500 pixels) was defined around each section (Figure 2.A). The image gray scale was then inverted to conform to the conventions of human functional-imaging data (Figure 2.B) and the background was subtracted using a rolling ball algorithm (Figure 2.C). A volume of interest was also drawn as an isocontour over the autoradiographic images to generate a mask of cerebral areas and to remove the possible contribution of adjacent sections (Figure 2. D)

In order to create a 3D image, all slices from each animal were imported as an image stack using PMOD (Figure 2.E). Adjacent sections were aligned using the PMOD Image Fusion tool. Due to the fact that the central slices provide the best quality, an appropriately positioned artifact-free central slice was selected as reference. The alignment of the whole volume was then performed sequentially between adjacent slices. To preserve the shape of the sections, a rigid transformation was chosen and the squared difference sum was used as the dissimilarity metric for optimization. After the automatic registration and visual inspection, a slight manual alignment was performed on some slices if necessary. Thus, an autoradiographic 3D volume was obtained for each animal (Figure 2.F). The fidelity of the alignment and 3D reconstruction process was assessed by visual inspection of internal structures viewed in different orthogonal sections, as well as by inspection of the smoothness of the cortex after rendering the surface.

The resultant 3D volume then had to be spatially normalized to a standard space (Figure 1). As a specific template of autoradiography does not exist, an MRI template was used. For each animal, a mask was applied over its corresponding MRI scan to remove the skull and scalp. Registration between the skull-stripped MRI scan and the 3D autoradiography image was performed automatically, followed by visual inspection and manual adjustment only if necessary. The previously calculated transformation matrix of MRI normalization was then applied to the autoradiographic 3D volume of the same animal (SPM8).

As previously done with the PET data, 3D autoradiographic volumes were smoothed with a Gaussian filter with a FWHM of 1 mm. As each animal is only acquired in one condition, a two sample t-test was performed in this case. Model parameters included global normalization, with proportional scaling and relative threshold masking at 80% of mean voxel value. Parameters were estimated and contrasts were generated to create statistical parametric maps of t-values. The statistical threshold was set at $p<0.005$ with an extent threshold of 100 contiguous voxels. Activated clusters were evaluated and anatomically located using a ROI template [27].

3. RESULTS

3.1. PET studies and 3D Autoradiography

PET scans from a representative rat under conscious and anesthetized conditions are shown in Figure 3.B and 3.C respectively, together with an MRI image (Figure 3.A). Widespread glucose hypometabolism can be seen when the animal was anesthetized versus conscious during the period of ^{18}F-FDG uptake.

Autoradiographic images were acquired, processed and successfully 3D reconstructed using the described procedure. Visual inspection of axial slices showed continuity of the borders of internal structures, such as the thalamus and basal ganglia. Each 3D-rendered surface also exhibited a consistent global

shape of the whole brain volume (Figure 4). The procedure was therefore well designed and produced geometrically consistent volumes.

Figure 5 illustrates representative autoradiographic coronal brain slices of conscious (Figure 5.B) and isoflurane-anesthetized animals (Figure 5.C). Under both conditions, glucose uptake was heterogeneously distributed according to regional differences in metabolic rate. As also noted with PET, a widespread decrease in glucose metabolism during anesthesia was clearly observed in the cortex, with hypometabolism also being apparent in subcortical structures.

3.2. Statistical analysis of PET studies

The areas displaying significantly decreased metabolism in the anesthetized versus the conscious condition are shown in Figure 6. A statistical t map was overlaid on the canonical MRI [10] coronal slices. Volume of interest contours of some cortical areas and the thalamus were superimposed on these images to show the location of the activation, resulting in the illustrated 3D volume-rendered image of the statistical t map.

An extensive cortical area showed significant differences with SPM analysis between the two acquisition conditions. SPM results are summarized in Table 1. One widespread cluster was detected, covering bilateral somatosensory cortex and other functional areas such as the auditory and visual cortices.

3.3. Statistical analysis of 3D Autoradiography

The results of the SPM analysis of the generated 3D autoradiography are shown in Figure 7. For visualization of the t-score map, significant voxels were projected onto the 3D-rendered high-resolution brain MRI scan, thus allowing anatomic identification. Based on a height threshold of $p<0.005$, there was significant deactivation in extensive cortical areas and the thalamus of anesthetized rats. Table 1 lists the activated clusters where significant differences were observed, together with the coordinates and significance level (Z and t scores and FWE-corrected p) of the maximum peak and the areas covered by each cluster.

4. DISCUSSION

In the present study, we explored the feasibility of using SPM for the analysis of ^{18}F-FDG PET of the rat brain acquired in our MOSAIC Philips small animal PET. Voxel-based analysis is a widely applied technique in human studies, and holds considerable promise for research using rodent models. Although several studies have performed SPM analysis in rats [28, 29], none of them has questioned the validity of the results taking into account the limitation in spatial resolution.

As our goal was to undertake a feasibility study, we chose the most widely-used radiotracer, ^{18}F-FDG, which measures glucose metabolism. The comparison selected was PET studies conducted in animals that were either conscious or maintained under isoflurane anaesthesia during the tracer uptake period, reflecting commonly encountered experimental protocols that have been reported to produce significantly different results. This issue has been

investigated in several animal species [13, 22, 30], although, to the best of our knowledge, no study to date has based its analysis on statistical maps.

In order to validate the capacity of our tomograph combined with a voxel based analysis to discriminate different conditions, results obtained from ^{18}F-FDG PET studies were compared to the autoradiographic data acquired under the same physiologic conditions, reconstructed as a 3D volume and analyzed using SPM. The use of a voxel-based technique to analyze PET images of rat brains would be validated if differences detected with ^{18}F-FDG PET matched our autoradiography measurements, which were considered to represent the gold standard.

Very few studies have compared the performance of PET and autoradiography in rodent brains, but in all cases in which this has been attempted the analysis was based on manually drawn ROIs. Toyama *et al.* [13] reported a high correlation between regional cerebral glucose quantified with ^{18}F-FDG PET and autoradiographic 2-^{14}C-DG in normoglycemic mice. Similarly, Mizuma *et al.* [30] found a clear correlation between regional uptake of ^{18}F-FDG in mice measured by PET and *ex vivo* autoradiography. In another study, Strome *et al.* [14] reported a significant correlation between *in vivo* small animal PET images and *in vitro* autoradiography measurements of striatal ^{11}C-DTBZ binding in the rat brain. However, none of these articles comparing PET and autoradiographic techniques performed voxel-based analysis. Therefore, the major novelty of this work is the validation of SPM analysis of rat brain PET by direct comparison to voxel-based analysis applied over high-resolution autoradiography.

Although the PET and autoradiography studies were carried out at different times, we rigorously applied the same protocols in relation to the animal's condition. Given that autoradiography is a post-mortem technique, each animal could only be studied under one condition, whereas both conditions were evaluated for each animal using PET. This difference required different statistical analysis, i.e., a paired t-test for PET and an unpaired t-test for autoradiography. The other crucial difference between autoradiography and PET is that the former is a 2D technique whereas PET is a 3D technique. However, this problem was solved with the construction of a 3D volume from the autoradiographic slices. Although this procedure has already been reported in the literature, with high quality results, the existing published images are mainly based on ^{14}C-autoradiography [17, 19], which yields better spatial resolution. Another noteworthy contribution of the present study is the demonstration that 3D volume construction also works for PET radiotracers, with the advantage that the same tracer (^{18}F-FDG) could be used for both techniques and a shorter film exposure was required for autoradiography (1 hour *vs.* several days).

The results obtained using SPM analysis of autoradiography revealed that rats anesthetized with 2% isoflurane had dramatically lower ^{18}F-FDG uptake in the cortex and the thalamus, compared with conscious animals, consistent with previous reports. Shimoji *et al.* [22] performed long-duration post-mortem PET on only one rat per condition, conscious and under isoflurane anesthesia during tracer uptake. Their images demonstrated visual heterogeneity between the

gray matter and the white matter of the conscious rat, whereas the anesthetized rat showed decreased activity and decreased heterogeneity in almost all regions. The same pattern was observed in our autoradiographic images, and similar results have also been published for mice. Mizuma *et al.* [30] quantitatively–evaluated ^{18}F-FDG PET results obtained under both conscious and anesthetized (2% isoflurane) conditions in mice and demonstrated that anesthesia lowered glucose consumption in the cerebral cortex, striatum and thalamus and, to a lesser extent, the cerebellum. We obtained similar results with regards to the cortex and thalamus, but did not observe any decrease in the striatum. We were also unable to analyze cerebellar uptake due to the fact that the autoradiographic slices at this level were of poor quality and therefore unsuitable for the 3D volume construction. Consequently, we only analyzed from frontal association cortex (Bregma 5.6 mm) to the vicinity of the inferior colliculus (Bregma -8.0 mm). Another study [13] evaluated metabolic effects of anesthesia in mice based on PET and autoradiography and found major quantitative differences in regional cerebral glucose utilization in the frontal, parietal, temporal and occipital cortices. Both Mizuma *et al.* and Toyama *et al.* performed their analyses based on manually drawn ROIs.

Voxel-based analysis of our PET data revealed significantly decreased ^{18}F-FDG metabolism in the anesthetized versus conscious condition. This difference principally occurred in sensorimotor cortex, reflecting the results obtained by autoradiography. However, autoradiography also revealed a significant deactivation in the thalamus that was not observed using PET. The reason for this may be the limited resolution of our PET tomograph which hindered

analysis on a voxel basis. However, the volume of the rat brain is approximately 2 cm^3 [31], meaning that even with the best dedicated PET tomograph with a spatial resolution of around 1.5 mm, the number of voxels inside the brain would be limited to a few hundred. Thus, the spatial resolution of current small animal PET systems imposes a major limitation for PET imaging studies of the rat brain. Despite this, the methodology still proved useful to highlight differences in the cortex, but was not sufficient to detect changes in deeper brain structures.

The limited resolution of our system produced partial volume effects, which means that the radioactive concentration of a structure spreads into the neighbouring tissues (spill-out), in addition to spread from these surrounding tissues into the structure under consideration (spill-in). These phenomena have been theoretically proven to prevent the correct quantification of 3D spheres with diameters less than 2.7 times the resolution of the acquisition equipment, measured as the FWHM of the point spread function [32]. In this particular study, using the Philips MOSAIC small animal PET tomograph, accurate quantification of structures smaller than approximately 8 mm was not feasible, which is greater than the size of some structures in the rat brain. This fact can be observed visually over our PET images, in which it is almost impossible to differentiate inner structures. Although pixel-based partial volume correction could restore activity in small structures, and several algorithms have been developed to correct this effect in human brain imaging [33-35], they need to be optimized for their application in rodents.

Partial volume effect is especially important when using a non-specific radiotracer such as ^{18}F-FDG, which measures the metabolism of glucose, the

metabolic substrate of most brain structures. However, when other tracers with spatially limited and specific uptake are used, it is possible to see and quantify the uptake in small regions such as the striatum [3, 36, 37]. In this case, analysis using ROIs or voxel-based techniques would be easier than with ^{18}F-FDG, although accurate quantification would still be limited by partial volume effects.

Despite the limitations of the present study, SPM analysis of ^{18}F-FDG PET acquired in our tomograph did prove useful for detecting major differences in extensive areas of the brain. It must be noted, however, that although a global decrease in cortical metabolism was apparent in our PET images, it was virtually impossible to anatomically localize the affected areas. Although the partial volume effect prevented detection of differences in smaller structures, SPM analysis of rat brain PET provides a good alternative to volume of interest analysis, particularly when taking into account that brain structures were visually indistinguishable over the PET image. The technique proposed in this paper can be useful to study several paradigms such as brain glucose metabolism in rat models of ischemia or neurodegenerative diseases.

5. CONCLUSIONS

The purpose of the present study was to examine the feasibility of using ^{18}F-FDG images of the rat brain acquired on a small animal PET tomograph that was not specifically designed for rodents in order to perform voxel-based analysis with SPM. Our results showed that regional differences identified by PET are in good agreement with those obtained using the standard reference

technique, 3D autoradiography, thereby confirming that any difference detected with PET actually exists. However, SPM analysis of 3D autoradiography data also highlighted changes in other small areas not detected with PET. Based on these experiments, even using a tomograph with limited spatial resolution, SPM analysis of ^{18}F-FDG PET of rat brain provides reliable results, although partial volume effects might make it difficult to detect slight differences in small regions. The procedures described in this study could also be applied to PET studies for other radiotracers and animal models.

7. FIGURE LEGENDS

Figure 1: Methodology for the spatial processing of ^{18}F-PET and 3D autoradiography to obtain the images to perform SPM analysis

Figure 2: Autoradiographic processing for 3D volume construction. A) Original digital slicesB.) Conversion of gray scale to inverted color scale   C.) Background subtraction D.) Application of a mask to eliminate adjacent sections E.) Image stack creation F.) Alignment and registration of the slices for definitive 3D volume creation.

Figure 3: Coronal slices of a MRI scan (A) together with PET data from a rat in the conscious (B) and anesthetized (C) state.

Figure 4: Evaluation of inter-slice registration shown in a 3D-rendered surface and in axial slices. Images before (A) and after (B) registration.

Figure 5: Coronal slices of a MRI scan (A) together with autoradiographic data from a rat in the conscious (B) and anesthetized (C) state. Autoradiographic data are normalized to the mean uptake in the whole brain for illustration purposes.

Figure 6: Statistical t map of SPM PET analysis showing hypometabolism in anesthetized rats overlaid on a canonical MRI scan. A) Coronal slices with superimposed volumes of interest. B) 3D volume-rendered image.

Figure 7: Statistical t map of SPM analysis of 3D autoradiography showing hypometabolism in anesthetized rats overlaid on a canonical MRI scan. A) Coronal slices with superimposed volumes of interest. B) 3D volume-rendered image.

8. TABLES

Table1: SPM analysis of 3D autoradiography and PET: Areas with decreased metabolism of anesthetized rats compared with conscious animals.

Cluster		Maximum voxel				Anatomical location	
N	Size (voxels)	Location (x y z)	p_{FWE}	T	Z	Anatomical Area	% of cluster in each region
PET							
1	42312	-7.0 3.8 -5.6	0.0052	13.4	5.1	Somatosensory Cortex R	25.0
						Somatosensory Cortex L	20.5
						Auditory Cortex R	8.9
						Visual Cortex R	5.9
						Others	<5
Autoradiography							
1	7703	-4.0 1.0 -6.6	0.00416	16.4	5.4	Somatosensory Cortex R	65.4
						Auditory Cortex R	13.1
						Visual Cortex R	9.4
						Other	<5
2	6109	5.0 1.2 -3.6	0.00748	15.3	5.3	Somatosensory Cortex L	81.0
						Auditory Cortex L	8.5
						Others	<5
3	2816	2.8 5.6 -4.6	0.02334	13.4	5.1	Thalamus L	33.6
						Thalamus R	30

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9. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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