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In Vivo Evaluation of Brain [^{18}F]F-FDG Uptake Pattern Under Different Anaesthesia Protocols

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Abstract. *Background/Aim: Since the use of anaesthetics has the drawback of altering radiotracer distribution, preclinical positron emission tomography (PET) imaging findings of anaesthetised animals must be carefully handled. This study aimed at assessing the cerebral [^{18}F]F-FDG uptake pattern in healthy Wistar rats under four different anaesthesia protocols using microPET/magnetic resonance imaging (MRI) examinations. Materials and Methods: Post-injection of 15±1.2 MBq of [^{18}F]F-FDG, either while awake or during the isoflurane-induced incubation phase was applied. Prior to microPET/MRI imaging, one group of the rats was subjected to forane-only anaesthesia while the other group was anaesthetised with the co-administration of forane and dexmedetomidine/Dexdor®. Results: While as for the whole brain it was the addition of dexmedetomidine/Dexdor® to the anaesthesia protocol that generated the differences between the radiotracer concentrations of the investigated groups, regarding the cortex, the [^{18}F]F-FDG accumulation was rather affected by the way of incubation. To ensure the most consistent and highest uptake, forane-induced anaesthesia coupled with an awake uptake condition seemed to be most suitable method of anaesthetisation for cerebral metabolic assessment. Diminished whole brain and cortical tracer accumulation detected upon dexmedetomidine/Dexdor® administration highlights the significance of the mechanism of action of different anaesthetics on radiotracer pharmacokinetics. Conclusion: Overall, the standardization of PET protocols is of utmost importance to avoid the confounding factors derived from anaesthesia.*

Clinical translation of discoveries derived from preclinical studies and imaging play a central role in the characterisation of human diseases and the establishment of their therapies. Therefore, the use of small animal model systems that enable the *in vivo* evaluation of various physiological (*e.g.*, metabolism, excretion, digestion) or pathological (*e.g.*, tumor development, infection) processes as well as the development of new diagnostic probes and therapeutic drug candidates is gaining increasing attention (1, 2).

Since positron emission tomography (PET) provides a precious mean for the three-dimensional, non-invasive, and quantitative assessment of radiopharmaceutical distribution both under physiological conditions and in various disease states, it is regarded as the mainstay diagnostic tool in preclinical sciences (3). Among the currently available radiopharmaceuticals, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]F-FDG) is by far the most broadly used in *in vivo* PET imaging (4). In addition, coupling microPET acquisition with either micro-computed tomography (microCT) or micro-magnetic resonance imaging (microMRI) makes the real-time and simultaneous assessment of functional and high-resolution morphological data possible (5).

Although preclinical examinations could be performed with different experimental animals such as mice, rats, cats,

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pigs, dogs or even non-human primates, the laboratory mice (Mus musculus) are by far the most popular animals in biomedical research due to their biological similarity to humans, low sustainability costs, rapid reproduction rate, easy handling, and the groundbreaking results derived from gene manipulation studies (6). To obtain reliable and reproducible results, animals must be immobilized during PET acquisition to avoid the development of motion artefacts (1). Furthermore, since stress and anxiety could also influence the outcome of PET neuroimaging studies, animal welfare/comfort/well-being must be ensured throughout imaging (7). For this reason, preclinical PET trials are generally performed under general anaesthesia (1, 8). An ideal anaesthetic agent is easy to administer, provides satisfactory immobilization within a short period of time, has limited adverse health ramifications, and exerts reversible and safe effects both on the animals and on the health care staff. Generally, propofol, ketamine and isoflurane are employed to achieve complete immobilization of the experimental animals (9). Although, the application of these agents has the drawback of affecting the homeostasis, the body temperature, cerebral blood perfusion, and metabolism of the small animals that may lead to subsequent alterations in the pharmacokinetics of the PET radiotracers (10-13).

Owing to the rapid anaesthetic induction and recovery, lack of organ toxicity, and the satisfactory control of the depth and duration of anaesthesia, isoflurane is the gold standard volatile anaesthetic in preclinical PET imaging (9, 11, 14-17). Of note, its meaningful systemic vasodilator effect is manifested at the level of the brain vasculature as well resulting in the enhancement of cerebral blood flow (CBF) and the disturbance of central autoregulation (18, 19). Considering that these effects may possibly alter the cerebral uptake kinetics of [^{18}F]F-FDG, we must exercise caution in the interpretation of the results derived from the brain PET studies of isoflurane-anaesthetized small animals (8, 11). Consequently, the evaluation of the effects of different anaesthetics on PET imaging has recently become the focus of exhaustive investigation (20-23).

Dexmedetomidine (Dexdor®) acting *via* the alpha-2 adrenergic receptors of vascular smooth muscle cells, exerts vasoconstrictive effects on cerebral blood vessels (24). Since Dexdor® alleviates vascular resistance and reduces CBF, this drug could be effectively used to compensate for cerebrovascular dilation generated by systemic isoflurane application (25). Hence, the co-administration of Dexdor® and isoflurane may provide the correct distribution of [^{18}F]F-FDG in the brain.

The present study was designed to analyse and compare the changes of cerebral [^{18}F]F-FDG uptake in rats anesthetized using isoflurane alone and the combination of isoflurane and dexmedetomidine/Dexdor® before applying PET/MRI imaging. Moreover, we intended to elaborate rat brain [^{18}F]F-FDG measurement protocols for preclinical PET/MRI examinations.

Materials and Methods

Animal housing. Inbred male, Wistar rats (n=20) of 150-200 grams were used in the present study (Charles River Laboratories, Sulzfeld, Germany). The experimental animals were bred and maintained under conventional laboratory conditions in individually ventilated cages (Sealsafe Blue line IVC system, Techniplast, Akronom Ltd., Budapest, Hungary). The temperature in the housing facility was maintained at 26±2˚C with a humidity of 50±10% throughout the whole experiment. Laboratory circadian cycle of 12 h was ensured. All experimental rats were provided SDS VRF1 rodent chow (Akronom Ltd.) and sterile drinking water *ad libitum*. All procedures complied with all applicable sections of the Hungarian Laws of XXVIII/1998, LXVII/2002, and the Laws of 2013 on the protection and welfare of the animals as well as the directions and regulations of the European Union. Ethical permission was granted by the Ethics Committee for Animal Experimentation of the University of Debrecen (ethical permission number: 14/2020/DEMÁB). The 3R policy was implemented.

Radiopharmaceutical synthesis. [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]F-FDG) is routinely produced in the radiochemical laboratory of the Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, Faculty of Medicine, University of Debrecen (Debrecen, Hungary) for human PET imaging in accordance with Good Manufacturing Practice (GMP) regulations.

In vivo positron emission tomography/magnetic resonance imaging (PET/MRI). All experimental rats were anaesthetized with a dedicated small animal inhalation anaesthesia device applying 1.5% isoflurane (Forane), 0.4 l/min O_2 and 1.2 l/min N_2O during radiotracer injection. 15±1.2 MBq of [^{18}F]F-FDG was intravenously *(iv.)* administered to the animals *via* the lateral tail vein in a volume of 150 μl in physiological saline (Salsol, TEVA, Debrecen, Hungary). Forty minutes after radiotracer injection, *in vivo* static brain PET/MRI acquisition was performed using the preclinical nanoScanPET/MRI system (Mediso Ltd., Budapest, Hungary). To maintain the appropriate body temperature of the rats and to ensure the continuous flow of the anaesthetic gases, special rat beds were used during the examination (MultiCell Imaging Chamber, Mediso Ltd.). In addition, T1-weighted MRI images (GRE EXT multi-FOV; Phase: 144; TR/TE 15/2 ms; FOV 60 mm; number of excitations: 2) were acquired for the precise anatomic localization of the different organs and the tissues.

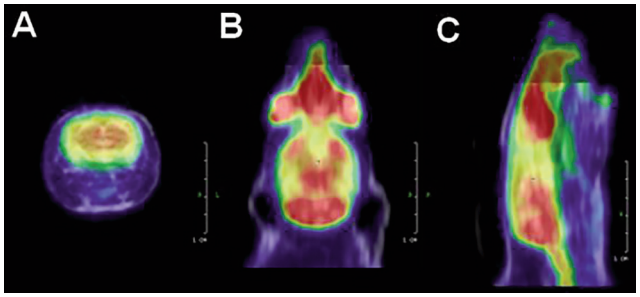

Figure 1. *Representative decay-corrected transaxial (A), coronal (B) and sagittal (C) positron emission tomography/magnetic resonance imaging (PET/MRI) images of the brain obtained from the forane/isoflurane (F)+F group 40 min after the intravenous administration of [^{18}F]2-fluoro-2-deoxy-D-glucose under isoflurane anaesthesia. Isoflurane-based anaesthesia was applied both during the incubation time and the in vivo PET/MRI imaging.*

PET data analysis. The PET images were reconstructed using three dimensional ordered-subsets-expectation-maximization (3D-OSEM) iterative image reconstruction/algorithm (Tera-Tomo, Mediso Ltd., Budapest, Hungary). Both the PET and the MRI images were automatically co-registered by the acquisition software (Nucline) of the PET/MRI device. We utilized the InterView™ FUSION image analysis software (Mediso Ltd., Budapest, Hungary) for the assessment of the reconstructed and registered PET/MRI images.

To determine [^{18}F]F-FDG accumulation in the investigated brain regions, volume of interests (VOIs) were manually drawn around selected cerebral areas applying the InterView™ FUSION software (Mediso Ltd.). The extent of [^{18}F]F-FDG uptake was expressed in standardized uptake values (SUV) that refers to the radiopharmaceutical accumulation within the VOI divided by the quotient of the administered dose and the weight of the animal. We used the following formula for the determination of this value:

[VOI activity (Bq/ml)]/[injected activity (MBq)/
animal weight (g)].

Standard deviation (SD) and different SUV values were calculated on the basis of the 3D VOIs: SUV_{mean}, maximum (SUV_{max}) and minimum (SUV_{min}) SUV values. SUV_{mean} is the average radiotracer concentration in a VOI, and SUV_{max} indicates the highest radiopharmaceutical activity within the VOI.

Anaesthesia protocols. Forane-induced anaesthesia throughout the whole experiment (F+F group). Regarding the first protocol, inhalation anaesthesia (1.5% isoflurane-forane and 0.4 l/min O_2 and 1.2 l/min N_2O) was induced in parallel with [^{18}F]F-FDG injection, and the experimental animals remained under forane anaesthesia until the termination of the examination, which means that forane-based anaesthesia was applied both during the radiopharmaceutical uptake period (incubation time) and the *in vivo* PET/MRI imaging.

Incubation with Forane anaesthesia, and examination under Forane/Dexdor®-induced anaesthesia (F+F/D group). Inhalation anaesthesia (1.5% isoflurane-forane and 0.4 l/min O_2 and 1.2 l/min N_2O) was administered to the study animals *via* intraperitoneal *(ip.)* injection of a bolus of dexmedetomidine (DEXDOR) at a concentration of 0.05 ml/100 g. Ten minutes after the injection of the bolus, we progressively reduced the level of isoflurane to 0.5-0.4%. Meanwhile, [^{18}F]F-FDG was *iv.* injected, and this "hybrid" type of anaesthesia was maintained throughout the rest of the experiment.

Incubation awake, and examination under Forane-induced anaesthesia (A+F group). Regarding the 3rd protocol, we applied a short period of inhalation anaesthesia (1.5% isoflurane-forane and 0.4 l/min O_2 and 1.2 l/min N_2O) during [^{18}F]F-FDG injection. Then, following a conscious incubation period prior to imaging, the study rats were re-anaesthetised using 1.5% isoflurane-forane and 0.4 l/min O_2 and 1.2 l/min N_2O.

Incubation awake, examination with Forane and Dexdor® administration (A+F/D group). Similarly to the previously described protocol, the rats were *iv.* injected with [^{18}F]F-FDG under inhalation anaesthesia (1.5% isoflurane-forane and 0.4 l/min O_2 and 1.2 l/min N_2O). Thereafter, an awake uptake phase was maintained. Following re-anaesthesia with 1.5% isoflurane-forane, 0.4 l/min O_2 and 1.2 l/min N_2O, a bolus of Dexdor was *ip*. given to the animals (concentration: 0.05 ml/100 g) and 10 min after its administration the level of isoflurane was gradually reduced to 0.5-0.4% to perform *in vivo* PET/MRI examinations.

Statistical analyses. F-probe and Student's *t*-test were applied for the statistical evaluation and the comparison of the obtained data. Results are shown as the mean±SD. The significance was set at $p<0.05$, unless otherwise indicated.

Results

Taking into account that the cerebral [^{18}F]F-FDG uptake is influenced by the incubation conditions and the method of anaesthesia, the optimization of measurement conditions was essential to ensure the appropriate evaluation of [^{18}F]F-FDG accumulation in the brain of the experimental rats.

Quantitative assessment of the cerebral [^{18}F]F-FDG uptake following different methods of anaesthetisation. Forane-

Table I. *Quantitative data analysis of the decay-corrected brain positron emission tomography/magnetic resonance imaging of the experimental animals (n=5) of the F+F group.*

	SUV_{mean}	SUV_{max}
Whole brain	5.52±0.81	8.14±0.93
Cortex	3.15±0.48	3.85±0.29

F: Forane/isoflurane; max: maximum; SUV: standardized uptake value.

Table II. *Quantitative data analysis of the decay-corrected brain positron emission tomography/magnetic resonance imaging images of the experimental animals (n=5) of the F+F/D group.*

	SUV_{mean}	SUV_{max}
Whole brain	4.12±0.42	6.83±0.86
Cortex	2.75±0.42	3.11±0.18

D: Dexmedetomidine/Dexdor®; F: forane/isoflurane; max: maximum; SUV: standardized uptake value.

induced anaesthesia throughout the whole experiment (F+F group). Figure 1 displays the decay-corrected representative brain PET/MRI scans (transaxial, coronal, and sagittal orientations) obtained from rats who were anaesthetized with isoflurane both during the radiopharmaceutical uptake period (incubation) and the *in vivo* imaging. Upon visual assessment, homogenous cerebral [^{18}F]F-FDG accumulation (Figure 1) could be detected, indicating that the application of forane throughout the incubation and the examination allows for the correct identification of the whole brain. In addition, compared to the other currently assessed anaesthesia methods, this protocol (F+F) provided the best visualization of the whole brain region. The quantitative PET parameters (SUV_{max} and SUV_{mean}) of these representative images are presented in Table I (SUV_{max} and SUV_{mean}: 8.14±0.93 and 5.52±0.81, respectively for the whole brain, and 3.85±0.29 and 3.15±0.48, respectively for the cortex).

Forane and Dexdor-induced anaesthesia throughout the whole experiment (F+F/D group). The decay-corrected, representative brain PET/MRI scans of the study rats of the F+F/D group are presented in Figure 2. The qualitative analysis of the PET scans revealed that in comparison with other ways of anaesthetisation, this protocol generated the lowest [^{18}F]F-FDG uptake both in case of the whole brain and the cerebral cortex.

Table II shows the SUV_{max} (6.83±0.86 and 3.11±0.18 for the whole brain and the cortex, respectively) and the SUV_{mean} (4.12±0.42 and 2.75±0.42 for the whole brain and the cortex, respectively) values measured in the PET/MRI scans of the F+F/D group.

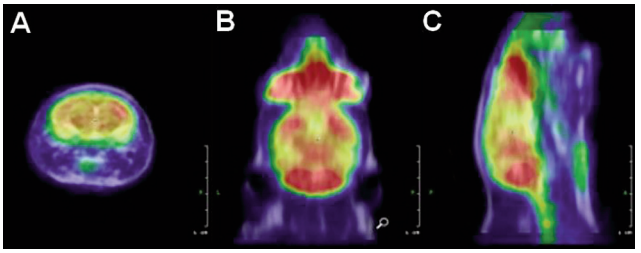

Figure 2. *Representative decay-corrected transaxial (A), coronal (B) and sagittal (C) positron emission tomography/magnetic resonance imaging (PET/MRI) images of the brain obtained from the forane/isoflurane (F+F)/dexmedetomidine/Dexdor® group 40 min after the intravenous administration of [^{18}F]2-fluoro-2-deoxy-D-glucose under isoflurane anaesthesia. Isoflurane-based anaesthesia was applied both during the incubation time and the in vivo PET/MRI imaging.*

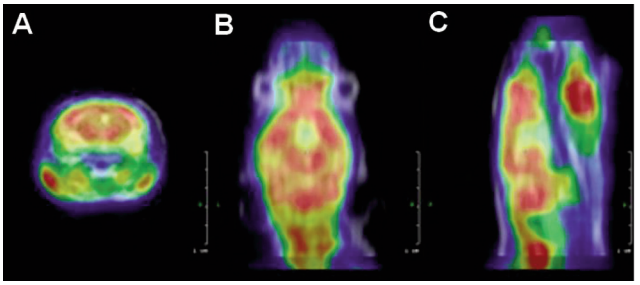

Figure 3. *Representative decay-corrected transaxial (A), coronal (B) and sagittal (C) positron emission tomography/magnetic resonance imaging (PET/MRI) images of the brain obtained from the awake+forane/isoflurane (A+F) group 40 min after the intravenous administration of [^{18}F]2-fluoro-2-deoxy-D-glucose under isoflurane anaesthesia. Isoflurane-based anaesthesia was applied both during the incubation time and the in vivo PET/MRI imaging.*

Incubation while awake, examination with Forane administration (A+F group). The decay-corrected representative brain PET/MRI scans of the rats of the A+F group are demonstrated in Figure 3. Compared to the other three anaesthesia methods, we found the highest cortical uptake using the A+F protocol.

Table III displays the quantitative analysis of the PET scans obtained from the A+F group. SUV_{max} values of 7.41±0.76 and 5.87±0.29 were registered for the whole brain and the cortex; respectively. In addition, the whole brain and the cortex could be characterised by respective SUV_{mean} figures being 5.27±0.67 and 5.01±0.63.

Incubation while awake, examination with Forane and Dexdor administration (A+F/D group). Figure 4 shows the decay-corrected representative brain PET/MRI images of

Table III. *Quantitative data analysis of the decay-corrected brain positron emission tomography/magnetic resonance imaging images of the experimental animals (n=5) of the A+F group.*

	SUV_{mean}	SUV_{max}
Whole brain	5.27±0.67	7.41±0.76
Cortex	5.01±0.63	5.87±0.29

A: Awake; F: forane/isoflurane; max: maximum; SUV: standardized uptake value.

Table IV. *Quantitative data analysis of the decay-corrected brain positron emission tomography/magnetic resonance imaging of the experimental animals (n=5) of the A+F/D group.*

	SUV_{mean}	SUV_{max}
Whole brain	4.22±0.63	6.32±0.51
Cortex	3.87±0.39	5.33±0.36

A: Awake; D: dexmedetomidine/Dexdor®; F: forane/isoflurane; max: maximum; SUV: standardized uptake value.

the experimental rats of the A+F/D group. In case of the whole brain, visually this anaesthesia protocol generated lower radioactivity than the A+F or the F+F ones. As indicated in Figure 4, the cortex is well delineated from the other cerebral regions.

The quantitative analysis of the PET images of the rats from the A+F/D group is exhibited in Table IV (SUV_{max} and SUV_{mean}: 6.32±0.51 and 4.22±0.63, respectively for the whole brain, and 5.33±0.36 and 3.87±0.39, respectively for the cortex).

Regional analyses of cerebral [^{18}F]F-FDG accumulation. Whole brain. As shown by the above indicated uptake values and images, as well as the summary diagram of Figure 5, the highest SUV_{mean} value was obtained for the whole brain when we applied forane during both the incubation as well as the imaging period (F+F SUV_{mean}: 5.52±0.81). Compared to the uptake values recorded in the F+F group, a slightly reduced radiotracer accumulation was experienced when the study rats were kept awake throughout the uptake phase, and forane-induced anaesthetisation was applied during the PET/MRI acquisition (A+F SUV_{mean}: 5.27±0.67). In contrast, the rats in this group that were awake during the incubation period and anaesthetized with the co-administration of forane and dexmedetomidine/Dexdor® (A+F/D SUV_{mean}: 4.22±0.63) exhibited markedly diminished [^{18}F]F-FDG uptake, and this was statistically significant. Finally, the lowest SUV_{mean} values were obtained when forane-generated incubation was applied

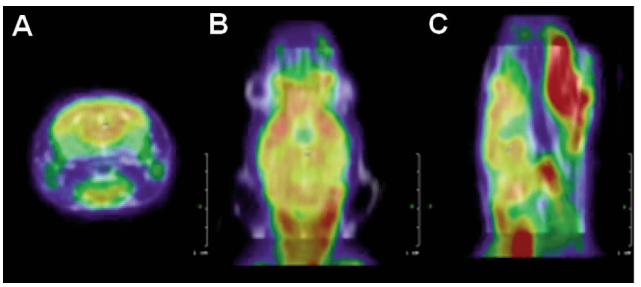

Figure 4. *Representative decay-corrected transaxial (A), coronal (B) and sagittal (C) positron emission tomography/magnetic resonance imaging (PET/MRI) images of the brain obtained from the A+F/D group 40 min after the intravenous administration of [^{18}F]2-fluoro-2-deoxy-D-glucose under isoflurane anaesthesia. Isoflurane-based anaesthesia was applied both during the incubation time and the in vivo PET/MRI imaging. A: Awake; D: dexmedetomidine/Dexdor®; F: forane/isoflurane; [^{18}F]F-FDG: [^{18}F]2-fluoro-2-deoxy-D-glucose.*

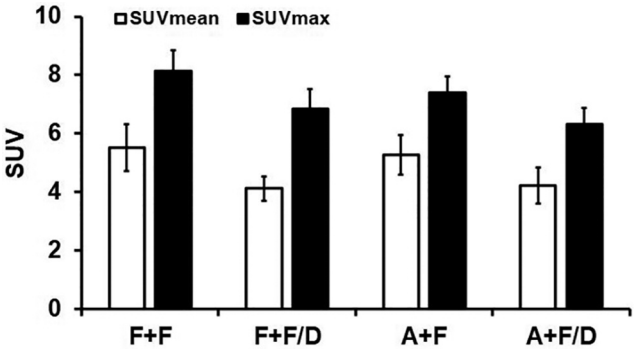

Figure 5. *Maximum (SUV_{max}) and mean (SUV_{mean}) [^{18}F]2-fluoro-2-deoxy-D-glucose uptake values of the whole brain measured under the assessed four different types of anaesthesia methods: 1) forane-incubated and forane-anaesthetised (F+F), 2) forane-incubated and anaesthetised under forane and dexmedetomidine/Dexdor® (F+F/D) 3) incubated awake and scanned under forane anaesthesia (A+F) 4) incubated awake and anaesthetised with forane and dexmedetomidine/Dexdor® (A+F/D). A: Awake; D: dexmedetomidine/Dexdor®; F: forane/isoflurane; max: maximum; SUV: standardized uptake value.*

followed by anaesthesia with the mixture of forane and dexmedetomidine/Dexdor® throughout the imaging period (F+F/D SUV_{mean}: 4.12±0.42).

Cerebral cortex. Upon the assessment of the accumulation values in the cortex, the [^{18}F]F-FDG uptake results were not necessarily in line with the SUV values obtained for the whole brain. Regarding the whole brain, the presence or the lack of dexmedetomidine/Dexdor® generated the differences between the radiotracer concentrations of the assessed groups, whereas as for the region of the cortex, the way of incubation (either incubation in an awake state or under

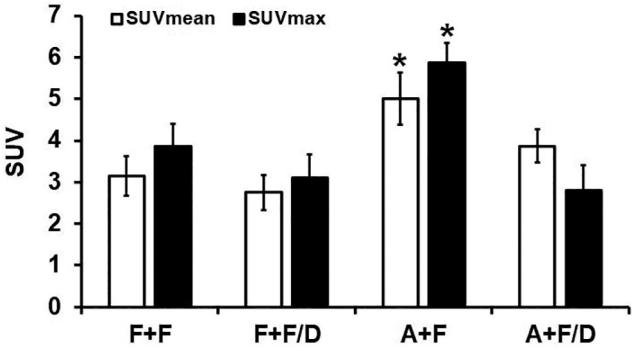

Figure 6. *Maximum (SUV_{max}) and mean (SUV_{mean}) [^{18}F]F-FDG uptake values obtained for the cerebral cortex under the four different anaesthesia protocols: 1) forane-incubated and forane-anaesthetised (F+F), 2) forane-incubated and anaesthetised under forane and dexmedetomidine/Dexdor® (F+F/D) 3) incubated awake and scanned under forane anaesthesia (A+F) 4) incubated awake and anaesthetised with forane and dexmedetomidine/Dexdor® (A+F/D). Significance level between the corresponding SUV data of A+F and the three other examined groups: p≤0.05. A: Awake; D: dexmedetomidine/Dexdor®; F: forane/isoflurane; [^{18}F]F-FDG: [^{18}F]2-fluoro-2-deoxy-D-glucose; max: maximum; SUV: standardized uptake value.*

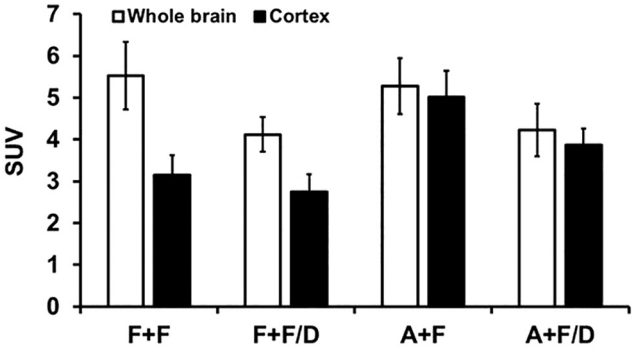

Figure 7. *Summary of [^{18}F]2-fluoro-2-deoxy-D-glucoseuptake in the whole brain and the cerebral cortex under four different types of anaesthesia protocols. A: Awake; D: dexmedetomidine/Dexdor®; F: forane/isoflurane; SUV: standardized uptake value.*

forane anaesthesia) affected the radiotracer accumulation. The highest mean uptake values were registered in the group incubated awake and anaesthetised exclusively with forane (A+F SUV_{mean}: 5.01±0.63). The incubation time spent awake, even with dexmedetomidine/Dexdor®-induced anaesthesia (A+F/D) did not reduce [^{18}F]F-FDG accumulation to the same extent as that in forane-incubated and forane-anaesthetised (F+F) or forane-incubated and forane+ dexmedetomidine/Dexdor®-anaesthetised (F+F/D) small animals. The corresponding SUV_{mean} values were 3.87±0.39, 3.15±0.48 and 2.75±042, respectively, for the A+F/D, F+F and F+F/D groups. The cortical radiotarcer accumulation values are displayed in Figure 6.

As presented in Figure 7, the most consistent and highest [^{18}F]F-FDG concentration was registered applying a conscious uptake phase and forane anaesthetisation. Consequently, the cerebral radiopharmaceutical uptake of the experimental animals could be best evaluated when an awake incubation phase is followed by forane-induced anaesthesia.

Discussion

PET has outstanding potential for advancing the understanding of the pathophysiology of human diseases, the development of diagnostic algorithms and treatment follow-up (26). Preclinical small animal PET studies represent the basis of translational research, as results derived from such experiments could be easily integrated into human patient care (3, 27). Since small animal brain [^{18}F]F-FDG accumulation examined by PET under isoflurane anaesthesia is markedly influenced by the applied anaesthetic agent, the results of cerebral radiopharmaceutical uptake may be misleading. To assess the influence of anaesthetic drugs on brain imaging we applied a hybrid isoflurane-Dexdor® anaesthesia protocol during PET/MRI acquisition.

Higher whole brain radiotracer accumulation detected upon isoflurane-induced incubation (SUV_{mean}: 5.52±0.81) - compared to incubation under awake condition - could possibly be attributed to isoflurane-related increase of CBF (23, 28). We hypothesize that more prominent CBF enhancement in association with isoflurane incubation could result in proportional elevation of [^{18}F]F-FDG uptake. Besides anaesthetics-related blood perfusion, however, several factors, including baseline serum glucose level, fasting state, muscular activity, body temperature, and various experimental circumstances such as anxiety or the way of radiopharmaceutical injection, influence the cerebral radiopharmaceutical accumulation that also need to be addressed for the proper interpretation of the uptake pattern (2, 29, 30). In addition, the different types of anaesthetics exert particular/specific effects on local neuronal activity as well as blood glucose concentrations that alter regional brain glucose metabolism (31-41).

Based on existing preclinical research data, isoflurane anaesthesia mainly results in diminished cerebral [^{18}F]F-FDG accumulation (42, 43). In a former dynamic [^{18}F]F-FDG PET study of Suzuki *et al.,* the whole-brain SUV value of the conscious group of animals (4.15±0.54) was significantly higher *(p<0.05)* relative to that of the isoflurane-anaesthetised (3.10±0.61) rats (4% isoflurane in 50% oxygen (1 l/min) and 1.5% isoflurane in 50% oxygen (1 l/min) for induction and maintenance, respectively) (23). Although their results seem to contradict ours, given the differences of the applied anaesthesia protocols between our study and that of Suzuki *et al*., as well as the fact that they performed dynamic scans, the findings of the two studies

could not really be compared. Confirmed by paired *t*-test, Spangler-Bickell *et al*. showed significantly reduced *(p*<0.05*)* isoflurane-generated radiotracer uptake of the whole brain in the group of the unconscious rats compared to the conscious ones, that contrasted with our observations (44). The research group of Spangler-Bickell *et al*. dealt with the assessment of the influence of isoflurane anaesthesia on the regional cerebral [^{18}F]F-FDG accumulation pattern of female Wistar rats (44). For this purpose, conscious (predominantly in an awake state and party under isoflurane anaesthesia) and unconscious (under isoflurane anaesthesia) PET examinations were performed. The former protocol meant that right after the radiotracer injection, a 60-75-min-long dynamic scan was performed in a conscious condition, followed by a 10-min-long static acquisition under isoflurane anaesthesia, and this anaesthetisation method was partly comparable to our A+F protocol. Identically to the F+F animal cohort, unconscious PET imaging was conducted by Spangler-Bickell *et al*. in the same group of rats, applying isoflurane anaesthetization prior to radiotracer injection that was maintained throughout the whole 75-min-long dynamic imaging. The contradiction between their and our findings may possibly be explained by the differences between the applied acquisition protocols, the way of anaesthesia and the method of [^{18}F]F-FDG administration (isoflurane: with a concentration of 1.5% in 0.4 l/min O_2 and 1.2 l/min N_2O *vs*. 2.5% in 2 l/min O_2 in the present work and in the study of Spangler-Bickell *et al*. (44), and [^{18}F]F-FDG: 15±1.2 MBq in a volume of 150 µl in physiological saline *via* the lateral tail vein *vs*. 20-30 MBq in a volume of 0.8 ml through an intra-femoral vein in the present work and in the study of Spangler-Bickell *et al*., respectively). Further, given that in the study of Spangler-Bickell *et al*. the dynamic PET acquisition started right after tracer injection, while in our study there was a 40-45-min long tracer distribution time prior to imaging, we speculate that the vasodilatory effect of isoflurane could not develop to such an extent to cause the increase of the radiopharmaceutical uptake.

Given the lower whole brain [^{18}F]F-FDG SUV values under the co-administration of isoflurane and Dexdor® compared to isoflurane alone anaesthesia, we suppose that Dexdor® reduced the vasodilator effects of isoflurane and related increased cerebral blood perfusion (SUV_{mean} F+F group: 5.52±0.81 *vs*. SUV_{mean} F+F/D group 4.12±0.42 and SUV_{mean} A+F group: 5.27±0.67 *vs*. SUV_{mean} A+F/D group: 4.22±0.63). Pioneering preclinical studies indicating the reduction of CBF in relation to dexmedetomidine may support our hypothesis (45, 46). Correspondingly, the application of sagittal sinus outflow technique in dogs resulted in diminished CBF following the addition of dexmedetomidine to either isoflurane or halothane anaesthesia (45, 46). Identically, the study of Zornow *et al*. revealed that the application of 10 µg/kg of dexmedetomidine generated a meaningful decrease (>45%) in CBF in isoflurane-anesthetized dogs, that also supports our assumption (46). Alike, Ohata *et al*. also observed a decrease in isoflurane-associated vasodilation upon dexmedetomidine administration (25).

According to a prior rat study, cyclic adenosine monophosphate (cAMP)-mediated protein kinase activation and subsequent calcium release is responsible for isoflurane-induced vascular dilation (47). In addition, ATP-sensitive K^+ channels triggered by isoflurane also have a role in the relaxation of cerebral blood vessels (48). In contrast, inhibitory membrane-bound guanine nucleotide-binding proteins that impede the overproduction of cAMP constitute the mechanism of action of alpha-2 agonist dexmedetomidine (Dexdor®) (49, 50). Additionally, dexmedetomidine (Dexdor®) exhibits direct vasoconstrictor effects by stimulating alpha-2 adrenergic receptors. Earlier studies in dogs also confirmed regulation of dexmedetomidine-related vasoconstriction by the alpha-2 adrenergic receptors of cerebrovascular smooth muscle cells (46, 51). Moreover, considering the findings of Asano *et al*., besides the stimulation of peripheral alpha-2 adrenergic receptors, the constrictor effects of dexmedetomidine are also conveyed *via* alpha-2-linked activation in distinct areas of the central nervous system (52). Furthermore, literature data indicate that dexmedetomidine also acts through the ATP-sensitive K^+ channels of the brain vessels (51). Upon stimulation with dexmedetomidine, the pancreatic alpha-2 adrenergic receptors impede insulin release leading to subsequent serum glucose level elevation that could also contribute to reduced radiotracer concentration *via* hyperglycaemia-mediated inhibition of [^{18}F]F-FDG uptake (53-55). So far, the exact mechanism underlying dexmedetomidine-associated [^{18}F]F-FDG uptake reductions is only poorly understood. We suppose that reduced cerebral vasodilation coupled with alpha-2 receptor-regulated hyperglycaemia may result in diminished radiotracer transport and cellular accumulation.

Applying isoflurane-incubation, reduced cortical metabolism was found either in the isoflurane or in the isoflurane/dexdor-anaesthetised groups (F+F and F+F/D) compared to the groups incubated awake (A+F and A+F/D). This is in line with the previous findings of Spangler-Bickell *et al*., who also noted the reduction of tracer accumulation in the cerebral cortex was related to isoflurane application in unconscious rats (44). On closer analyses of the [^{18}F]F-FDG PET images of healthy rats performed under continuous isoflurane administration or under awake uptake state, reduced cortical [^{18}F]F-FDG concentration was reported in the anaesthetised animal group by Jahreis *et al*., that agrees with our results (56). The activity of the conscious experimental animals during the awake uptake phase could partially explain the experienced results (57).

Upon the evaluation of the effects of isoflurane anaesthesia and *iv*. morphine self-administration (MSA) on the cerebral

glucose metabolism of adult male Sprague-Dawley rats, Park *et al*. noticed that isoflurane anaesthesia reduced metabolism in the cortex relative to the awake state, that corresponded to our findings registered in the A+F and the F+F groups (21). In their study, prior to [^{18}F]F-FDG PET/CT imaging, performed under isoflurane anaesthesia (4% induction, 1.5-2% maintenance), both the MSA and the control, saline self-administered (SSA/control) experimental rats, were either isoflurane-anaesthetised or were kept awake throughout the 45-min-long tracer distribution time. Beyond the diminished cortical [^{18}F]F-FDG accumulation, the following brain regions also demonstrated glucose hypometabolism: olfactory bulb, thalamus, basal ganglia, and the corpus callosum. To investigate the influence of anaesthesia on cerebral glucose metabolism Matsumura *et al*. analyzed the static PET images of 50 male Sprague-Dawley rats under different types of anaesthesia, including isoflurane (12). Followed by isoflurane-induced incubation (pre-[^{18}F]F-FDG-injection group) Matsumura *et al*. showed reduced cortical tracer concentration in comparison with that of the conscious control group (12). Identically, in a previous study of Mizuma *et al*., the regional cerebral glucose metabolic rate (rCMRglu) in the cortex was reduced by 66% upon isoflurane application (58).

Although the underlying mechanism behind isoflurane-triggered region-specific glucometabolic reduction has not been fully uncovered, altered glucose-regulated insulin secretion associated with isoflurane administration could contribute to the effect (59). Furthermore, the decreased tracer uptake could also be attributed to the impact of isoflurane on GABAergic neurons expressed at high levels in the cortex (60, 61). Apart from these, suppressed neuronal activity, hyperglycaemia-related saturation of glucose transporters at blood vessels as well as extra-intracellular barriers or changes in hexokinase activity may also explanation these observations (62, 63).

Similarly to the uptake figures of the whole brain, lower forane+Dexdor® anaesthesia-associated SUV values were registered in the cerebral cortex for the forane-incubated rats (F+F/D; SUV_{mean}: 2.75±042) than for the conscious animal group (A+F/D; SUV_{mean}: 3.87±0.39). Although, as far as we are aware, no prior studies dealt with the examination of the effects of the co-administration of dexmedetomidine and isoflurane on cerebral metabolism, Laaksonen *et al*. conducted an in-human PET study in which they compared the influence of dexmedetomidine on CMRglu with that of propofol, sevoflurane and S-ketamine in several brain areas including the prefrontal, the parietal, the lateral temporal, the lateral occipital and the entorhinal cortex (64). The lowest metabolic rate was registered under dexmedetomidine anaesthesia in almost all assessed brain regions, and statistically significant differences were found between the metabolism of nearly all cerebral areas upon dexmedetomidine application compared to the other anaesthetics. According to our results, we suppose that it was the way of incubation (awake or isoflurane-induced) that influenced the tracer accumulation in the cortex. It may probably be attributed to the fact that the temporal distribution of the radiopharmaceutical accumulation may show heterogeneity between the different brain areas, and the cortical region could be characterised by a relatively early tracer uptake. Therefore, the cortex possibly takes up most of the radiotracer during the incubation period.

Overall, given that the most consistent and highest [^{18}F]F-FDG uptake obtained during an awake incubation phase followed by forane-induced anaesthesia (A+F), we conclude that this method of anaesthetisation ensures the best method for the evaluation of the cerebral radiopharmaceutical uptake of the experimental animals. This could be ascribed to both hyperglycaemia and vascular dilation induced by dexmedetomidine/Dexdor®. Taking our results into account, the interpretation of the discoveries derived from the [^{18}F]F-FDG brain PET imaging of anaesthetized animals must be handled with caution. To overcome the misleading cerebral biosignals caused by the use of different anaesthetics, comprehensive future work is warranted that provide a deeper understating of the detailed effects of anaesthesia on [^{18}F]F-FDG pharmacokinetics.

Conclusion

In conclusion, applying microPET/MRI imaging on healthy Wistar rats, we demonstrated that anaesthetics altered cerebral [^{18}F]F-FDG uptake under four types of anaesthesia protocols. Based on our results, incubation awake along with forane anaesthesia provided the best conditions for cerebral biodistribution assessment. Diminished whole brain and cortical tracer accumulation detected upon dexmedetomidine/Dexdor® administration highlights the significance of the mechanism of action of different anaesthetics on radiotracer pharmacokinetics. Therefore, the choice of the most suitable anaesthesia method requires careful consideration to ensure correct evaluation of cerebral metabolism. Given that preclinical small animal model systems represent a cornerstone in biomedical research, the establishment of standardised protocols for the [^{18}F]F-FDG brain PET imaging of laboratory animals constitutes a research priority.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Zita Képes: Data Curation, Validation, Literature search, Writing original draft; Viktória Arató: Methodology, Investigation; Csaba

Csikos: Visualization; Éva Hegedűs: Investigation; Regina Esze: Investigation; Tamás Nagy: Methodology, Investigation; István Jószai: Methodology, Investigation; Miklós Emri: Methodology, Validation; István Kertész: Validation, Supervision; György Trencsényi: Data Curation, Visualization, Validation, Conceptualization, Supervision. All Authors have read and agreed to the published version of the manuscript.

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