


RESEARCH ARTICLE

Test-Retest Stability of Cerebral 2-Deoxy-2- $[^{18}\text{F}]$ Fluoro-D-Glucose ($[^{18}\text{F}]$ FDG) Positron Emission Tomography (PET) in Male and Female Rats

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Abstract

Purpose: An important issue in rodent imaging is the question whether a mixed population of male and female animals can be used rather than animals of a single sex. For this reason, the present study examined the test-retest stability of positron emission tomography (PET) with 2-deoxy-2- $[^{18}\text{F}]$ fluoro-D-glucose ($[^{18}\text{F}]$ FDG) in male rats and female rats at different phases of the estrous cycle.

Procedures: Long–Evans rats (age 1 year) were divided into three groups: (1) males ($n=6$), (2) females in metestrous (low estrogen levels, $n=9$), and (3) females in proestrous (high estrogen levels, $n=7$). Two standard $[^{18}\text{F}]$ FDG scans with rapid arterial blood sampling were made at an interval of 10 days in subjects anesthetized with isoflurane and oxygen. Body temperature, heart rate, and blood oxygenation were continuously monitored. Regional cerebral metabolic rates of glucose were calculated using a Patlak plot with plasma radioactivity as input function.

Results: Regional metabolic rate of glucose ($\text{rCMR}_{\text{glucose}}$) in male and female rats, or $[^{18}\text{F}]$ FDG uptake in females at proestrous and metestrous, was not significantly different, but females showed significantly higher standardized uptake values (SUVs) and Patlak flux than males, particularly in the initial scan. The relative difference between the scans and the test-retest variability (TRV) were greater in females than in males. Intra-class correlation coefficients (ICCs) of $\text{rCMR}_{\text{glucose}}$, SUV, normalized SUV, and glucose flux were good to excellent in males but poor to moderate in females.

Conclusions: Based on these data for $[^{18}\text{F}]$ FDG, the mixing of sexes in imaging studies of the rodent brain will result in an impaired test-retest stability of PET data and a need for larger group sizes to maintain statistical power in group comparisons. The observed differences between males and females do not indicate any specific gender difference in cerebral metabolism but are related to different levels of non-radioactive glucose in blood plasma during isoflurane anesthesia.

Key words: Small animal imaging, Regional cerebral metabolic rate of glucose, Gender differences, Test-retest stability, Estrogens

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Introduction

An important issue in rodent imaging is the question whether a mixed population of female and male animals

can be used, rather than animals from a single sex. The inclusion of both sexes in study protocols might lead to a reduction of the number of surplus animals since experimental animals of the currently undesired sex would no longer need to be excluded. In order to assess the impact of gender mixing on the variability of preclinical positron emission tomography (PET) data, we here report the results of repeated, quantitative 2-deoxy-2- ^{18}F fluoro-D-glucose (^{18}F FDG) scans of the brain of adult rats.

Many reports on gender differences of glucose metabolism in the human brain have been published in the past decades, but the results have been conflicting. Some studies reported that global metabolic rate in the female brain is higher than in males, and possibly related to estrogen levels [1–3]. Other studies noted the mentioned difference in patients during motor activity but not in healthy subjects [4], or observed no differences between the sexes [5–8].

When regional rather than global rates of glucose consumption were measured in the human brain, gender differences were frequently observed but the nature of these differences and the involved brain areas were divergent. According to one study, temporal-limbic regions and cerebellum consume more glucose in men than in women, whereas cingulate is more active in women than in men [9]. Other studies reported opposite findings: temporal poles and cerebellum being metabolically more active in females than in males [8], or posterior parts of the brain being more active in females whereas anterior brain is more active in males [10]. Voxel-based rather than region of interest (ROI)-based methods indicated that the male brain has significantly higher glucose consumption in the right insula, middle temporal gyrus, and medial frontal lobe, whereas the female brain has a significantly higher metabolic rate in the hypothalamus [11]. A study using statistical parametric mapping (SPM) reported that metabolism in visual cortex and cerebellum is significantly higher in males than in females [12].

When gender differences are studied in experimental animals rather than humans, the subjects can be scanned in a carefully controlled way: at the same age, at a well-defined phase of the estrous cycle, at similar levels of oxygenation, and in the anesthetized rather than the awake state. Inter-individual differences related to breathing, diet, or consciousness can thus be avoided, and observed differences in brain metabolism can be more easily related to other physiologic parameters. Test-retest studies of cerebral ^{18}F FDG-PET in rodents with arterial blood sampling have been performed by various groups, but only male animals were included in these studies [13, 14].

If global or regional differences in glucose metabolism, such as have been reported for the human brain, would also exist in rodents, gender mixing would result in significant increases of inter-individual variability in preclinical studies and would complicate the detection of group differences. Since age is known to have a significant (and possibly a gender-related) impact on cerebral metabolism [15] and

since glucose consumption in the brain may be positively correlated to estrogen levels [1], we planned our animal study in the following way: All animals were of the same age, and were either males or females at two different phases of the estrous cycle (proestrous or metestrous, *i.e.*, at high or low levels of circulating estrogens). All animals were scanned again after an interval of two estrous cycles, which is on average 10 days in a female rat. Our study aimed to examine the test-retest stability of ^{18}F FDG-PET in male and female rodents. Secondary research questions were as follows: Are gender differences of cerebral glucose metabolism detectable in rats, and is the cerebral uptake of ^{18}F FDG in female rats related to estrogen levels?

Materials and Methods

Reagents

^{18}F FDG was produced by the Hamacher method [16]. Radiochemical purity of the product was virtually 100 %.

Animals

The study protocol complied to European Directive 2010/63/EU and the Law on Animal Experiments of The Netherlands; it was approved by the Central Committee on Animal Experiments of The Netherlands (The Hague, license no. AVD105002015166) and the Institutional Animal Care and Use Committee of the University of Groningen (protocol 15166-01-001). The experiments described in this paper are reported in compliance with the ARRIVE guidelines. We used 22 Long–Evans rats (HsdBlu:LE, age 1 year) which were born at the Central Animal Laboratory of the University Medical Center Groningen. The animals were housed in Makrolon cages on a layer of wood shavings at a temperature of 21 ± 2 °C under a fixed 12-h light–dark regime. Standard laboratory chow (RMH-B, Hope Farms, The Netherlands) and water were available *ad libitum*. Information concerning the rats (body weights at the moments of scanning, injected tracer dose, blood glucose levels) is provided in Table 1. At the beginning of the study (day of the first PET scan), the animals were divided into three groups: (1) females at proestrous (high estrogen levels), (2) females at metestrous (low estrogen levels), and (3) males. The group size was estimated based on previously obtained ^{18}F FDG-PET data in ovariectomized female rats, treated with placebo or estradiol. Assuming an alpha of 0.05 and a power of 0.8, the calculated group size ranged from 5 to 10 depending on brain region and duration of estradiol treatment. For the first PET scan, male rats were randomly chosen. Random selection of all animals in this study was not possible since rats were selected for scanning based on their gender and (for females) the actual phase of the estrous cycle. When animals had been scanned for the first time, the date of their second scan was fixed.

Table 1.. Animal and scan data

Sex	Female	Male
Number of animals	16	6
Body weight (g) at scan 1	394 ± 54	658 ± 46*
Body weight (g) at scan 2	385 ± 49 ^S	636 ± 50* ^S
Injected dose (MBq) at scan 1	40.2 ± 3.7	37.5 ± 4.3
Injected dose (MBq) at scan 2	37.7 ± 7.1	36.0 ± 3.7
Cycle phase at scan 1	P (7 rats) E (0 rats) M (9 rats) D (0 rats)	n.a.
Cycle phase at scan 2	P (2 rats) E (2 rats) M (6 rats) D (6 rats)	n.a.
Blood glucose (mmol/l) at scan 1	11.0 ± 1.4	15.9 ± 2.8*
Blood glucose (mmol/l) at scan 2	10.7 ± 1.5	14.3 ± 2.1*
Interval between anesthesia induction and scan 1 (min)	51 ± 9	52 ± 6
Interval between anesthesia induction and scan 2 (min)	40 ± 8	43 ± 5
Interval between scans (days)	10.0 ± 0.4	10.5 ± 1.2

Data are reported as mean ± SD. Significant differences are indicated: * $P < 0.001$ (male vs. female), ^S $P < 0.001$ (retest vs. test)
P proestrous, *E* estrous, *M* metestrous, *D* diestrous, *n.a.* not applicable

Vaginal Smears

In order to determine the length of the estrous cycle in each female rat, vaginal smears were taken daily (before 10:00 h) for at least 15 consecutive days (about 3 cycles). An additional vaginal smear was taken on the day of the first PET scan. A maximum of 20 vaginal smears was obtained from a single rat, using an eyedropper tool. The eyedropper pipette was filled with 0.2 ml of saline and was inserted approximately 5 mm into the rat's vagina. The saline was quickly released from the dropper and then immediately drawn back into the pipette. Saline samples containing cells from the vaginal wall were placed on a microscope slide and were examined at ×100 magnification to determine the phase of the cycle [17].

PET Scanning

All PET scans were made between 11.30 and 14.00 h, *i.e.*, near the middle of the animal's 12-h daylight period. The investigators who made the scans (JWAS, AvW) were blinded for the estrous phase of female rats, which had been determined by JD. On the day of the first PET scan, rats were anesthetized with isoflurane in 95 % oxygen (5 % isoflurane for induction and 2 % for maintenance). A cannula was placed in a side branch of the femoral artery, using a surgical procedure which was developed in our laboratory and was published previously [18]. A second cannula was placed in a tail vein. The venous cannula was used for tracer injection and the arterial cannula for blood sampling during the scan. All surgical actions were performed under aseptic conditions. After this surgery, a small sample of blood was drawn from the arterial cannula. Blood glucose in this sample

was determined enzymatically, with hexokinase and glucose-6-phosphate dehydrogenase. The rat was then positioned in the small animal PET camera (Siemens/Concorde MicroPET Focus 220) with its brain in the field of view. A transmission scan of 515 s was made, using a ⁵⁷Co point source, for later correction of attenuation and scatter of 511 keV gamma radiation by tissue. Subsequently, the tracer [¹⁸F]FDG was injected during a period of 1 min, using an injection pump (Harvard Apparatus, Holliston, MA, USA). Data acquisition of the PET camera (list mode protocol) was started simultaneously with activation of the pump. During the PET scan of 60 min, 15 arterial blood samples (volume 0.1–0.15 ml) were taken at the following intervals: 10, 20, 30, 40, 50, 60, 90, 120, 180, 300, 450, 600, 900, 1800, and 3600 s. Using these samples, radioactivity in 25 µl of whole blood and 25 µl of plasma was determined by *ex vivo* gamma counting. Heating pads and electronic temperature controllers (M2M Imaging, Cleveland, OH, USA) were used to maintain the body temperature of the rat close to the normal value (*i.e.*, between 37 and 38 °C) during surgery and PET scanning. Body temperature of the animal was continuously registered, using a rectal PTC thermometer and a data logging system (PicoTechnology, St. Neots, UK). Heart rate and oxygen level of the blood were continuously monitored, using a pulse oximeter (PulseSense, Nonin Medical, Plymouth, MN, USA). Eye salve was applied to prevent dehydration of the cornea. After the PET scan, the cannulas were removed. The side branch of the femoral artery was closed and the surgical wound in the hind leg was stitched. Analgesia was applied to reduce animal discomfort (subdermal Marcaine 0.5 %, 2.5 mg/kg). Animals were returned to their (pre-warmed) home cages to wake up from anesthesia and were

allowed at least 9 days for recovery. During this period, they were examined at regular intervals to examine the status of the wound and to score their behavior and condition. Unexpected complications (uncontrollable bleeding, signs of paralysis, disturbed mobility, significant weight loss) were not encountered.

A second PET scan was made of each animal after an interval of 9 to 13 (in most cases 10) days, using the same procedure as described for scan 1, but after this scan, the animals were euthanized by extirpation of the heart, under deep isoflurane anesthesia.

Data Analysis

The list mode data of the emission scans were reframed into a dynamic sequence of 6×10 s, 4×30 s, 2×60 s, 1×120 s, 1×180 s, 4×300 s, and 3×600 s frames. The data were reconstructed per time frame, using an iterative reconstruction algorithm (ordered subsets expectation maximization, OSEM 2D with Fourier rebinning, 4 iterations and 16 subsets). The final datasets consisted of 95 slices with a slice thickness of 0.8 mm and an in-plane image matrix of 256×256 pixels. The voxel size was $0.63 \times 0.63 \times 0.80$ mm and the linear resolution at the center of the field of view 1.5 mm. Data sets were corrected for decay, random coincidences, scatter, and attenuation.

Independent female and male rat FDG-PET brain templates were constructed [19], each aligned to the same MRI template [20]. Individual rat brain images were aligned to the appropriate sex-specific template using rigid-body transformation and the software package PMOD (version 3.804). As confirmed by comparison on CT images, the male brains were about 5 % bigger than the brains of females. Accordingly, the ROIs were linearly scaled (5 %) to accommodate the bigger male brain. Nine ROIs, whole brain including cerebrospinal fluid space, cerebellum, mesencephalon, pons, striatum, cerebral cortex, hippocampus, hypothalamus, and thalamus, were defined based on the MRI template. Time-activity curves (TACs) were calculated for the entire brain and for these brain regions. Calculated SUVs of tracer uptake in these ROIs were either uncorrected or normalized for the blood glucose level, using the formula: $SUV_{corr} = [SUV \times \text{blood glucose (mmol/l)}] / [\text{Average blood glucose for the entire gender group}]$. Glucose influx rate (slope) and regional cerebral metabolic rates of glucose were calculated by Patlak graphical analysis [21, 22]. The fit was started at 10 min after tracer injection and the Akaike information criterion was smallest if cerebral blood volume was fixed at 5.0 %, a value reported in the literature [23]. Radioactivity in arterial plasma was used as input function, and radioactivity in whole blood was employed to estimate the contribution of radioactivity in blood to measured activity in the brain.

Statistics

The reproducibility of measurements performed in the same animal at test and retest was calculated as a relative difference (Eq. 1) and as TRV (Eq. 2): [24].

$$\text{Relative difference} = 100 * (\text{Retest} / \text{Test}) - 100 \quad (1)$$

$$\text{TRV} = 100 * [(\text{Test} - \text{Retest}) / ((\text{Test} + \text{Retest}) / 2)] \quad (2)$$

Measurement variability was expressed as coefficient of variance (COV; Eq. 3):

$$\text{COV} = 100 * (\text{Standard Deviation} / \text{Mean}) \quad (3)$$

The reliability of the measurements (mean square between subjects, MSBS, and mean square within subjects, MSWS) was expressed as ICC (Eq. 4):

$$\text{ICC} = [\text{MSBS} - \text{MSWS}] / [\text{MSBS} + (n - 1) * \text{MSWS}] \quad (4)$$

We used the two-way mixed model with the absolute agreement type and a confidence interval of 95 % to calculate ICC [25]. The generalized estimating equations model was used to account for the repeated measurements in the design and a few missing data for heart rate and blood oxygenation. The independent correlation matrix was selected for the analysis, and *P* values reported using the Wald test. *P* < 0.05, without correction for multiple comparisons, was considered statistically significant.

Results

Glucose Metabolic Rate and Flux

The $rCMR_{glucose}$ of whole brain was similar in female and male rats (18.3 ± 0.6 vs. 19.1 ± 2.0) but was significantly higher at scan 1 than at scan 2 (20.8 ± 1.4 vs. 16.6 ± 0.8 , *P* < 0.001). Similar, statistically significant differences between the scans were observed in all studied brain areas (see Fig. 1a and Supplementary Table 1). Significant differences in $rCMR_{glucose}$ of the female brain at proestrous and metestrous were not detected.

Glucose flux in whole brain was higher in females than in males (0.016 vs. 0.012 , *P* = 0.002) and was higher at scan 1 than at scan 2 (0.015 vs. 0.013 , *P* < 0.001). Similar, statistically significant, differences between the sexes and, for females, between the scans were observed in all studied brain areas (Fig. 1b and Supplementary Table 2 (see electronic supplementary material (ESM)). Significant

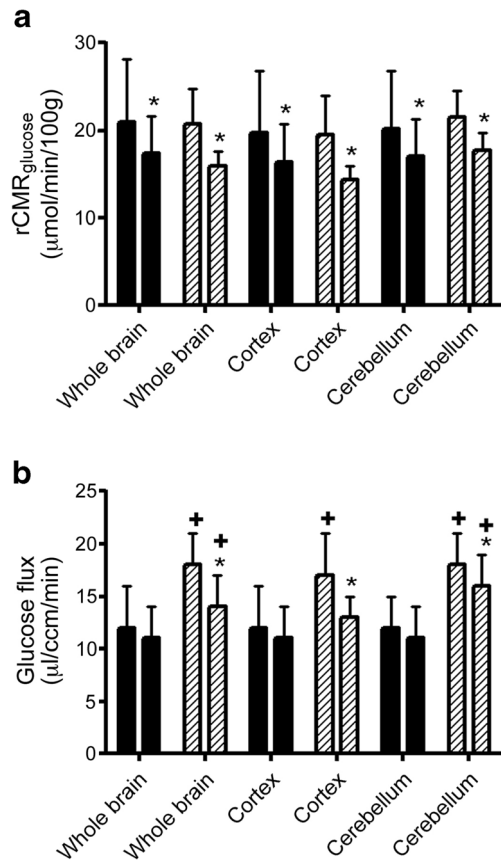


Fig. 1. **a** rCMR_{glucose} and **b** glucose flux in whole brain, cortex, and cerebellum at test and retest. Solid bars indicate data of male, and hatched bars data of female rats, plotted as mean \pm SD. * = Significantly different from the test value; + = significantly different from the corresponding value in males.

differences in regional glucose flux of female brain at proestrous and metestrous were not detected, with cerebellum as the only exception (0.019 vs. 0.016 , $P < 0.02$).

SUV and Normalized SUV

TACs of F-18 in whole brain and blood plasma after injection of [^{18}F]FDG are shown in Fig. 2. PET images of the brain of a representative female and male rat are presented in Fig. 3.

SUV values for whole brain were higher in females than in males (3.08 ± 0.15 vs. 2.46 ± 0.24 (Fig. 2a and Supplementary Table 3 in ESM) and were higher at scan 1 than at scan 2 (2.87 ± 0.15 vs. 2.66 ± 0.15 , $P < 0.05$). If smaller brain areas were analyzed, the same trends were observed but the sex difference reached statistical significance only in cerebellum, hypothalamus, mesencephalon, and pons and the scan difference in cortex, hippocampus, hypothalamus, mesencephalon, pons, and thalamus. Normalized SUV values for whole brain tended to be higher in females than

in males (3.07 ± 0.17 vs. 2.45 ± 0.27 , $P = 0.054$) and were higher at scan 1 than at scan 2 (2.95 ± 0.19 vs. 2.57 ± 0.15 , $P = 0.001$ (Fig. 2b and Supplementary Table 4 in ESM)). Significant differences between uncorrected or normalized SUV values for whole brain or for individual brain regions of female rats at proestrous and metestrous were not detected (Fig. 4).

In order to explore a factor which might affect apparent metabolic rates of glucose, we tested whether there was any significant correlation between [^{18}F]FDG uptake in the brain and body weight.

In the range of body weights examined in this study, we did not observe significant correlations between any calculated parameter of cerebral glucose uptake and body weight.

Physiological Parameters

An overview of some physiological parameters of the animals during the PET scans is provided in Fig. 5. Since no significant differences of body temperature, heart rate, or blood oxygenation were noted between scan 1 and scan 2, Fig. 5 shows pooled data from both scans. The core temperature of female rats (measured with a rectal PTC thermometer) was slightly higher than that of males (37.4 ± 0.1 vs. 36.8 ± 0.1 °C, mean \pm SEM, $P < 0.001$) and tended to rise during the scan (Fig. 5a). In contrast to the core temperature, the temperature of the extremities (not measured) seemed to drop more in females than in males, resulting in pale hind paws in females and occasional difficulties in recording oxygenation with a pulse oximeter. Heart rate of all animals declined during the scan, probably as a consequence of prolonged anesthesia. This effect of time was statistically significant ($P < 0.02$), but the difference between the sexes did not reach statistical significance, although heart rate tended to drop more in females than in males (Fig. 5b). Finally, male rats maintained higher oxygenation levels in their blood than females (95.8 ± 0.4 vs. 93.8 ± 0.4 %, $P = 0.001$, Fig. 5c). Although the observed differences were minor, the physiological condition of male rats during the scan appeared to be more stable than that of females.

Levels of blood glucose were 34 to 45 % higher in male rats than in females (Table 1) and were also higher in scan 1 than in scan 2 (overall 13.4 ± 0.6 vs. 12.5 ± 0.5 mg/ml, $P < 0.02$). At the age of 1 year that was chosen in this study, male rats were considerably heavier than females (Table 1). All animals lost some weight during the 10-day interval between the scans. The average loss was 9 g for females and 22 g for males, representing 2.3 to 3.3 % of their initial body weights.

For the test scan, we managed to select seven female rats in proestrous and nine females in metestrous (Table 1). Based on the length of the estrous cycle in each individual (which we had determined during a period of 15 days prior to scan 1), we expected a similar ratio of females in

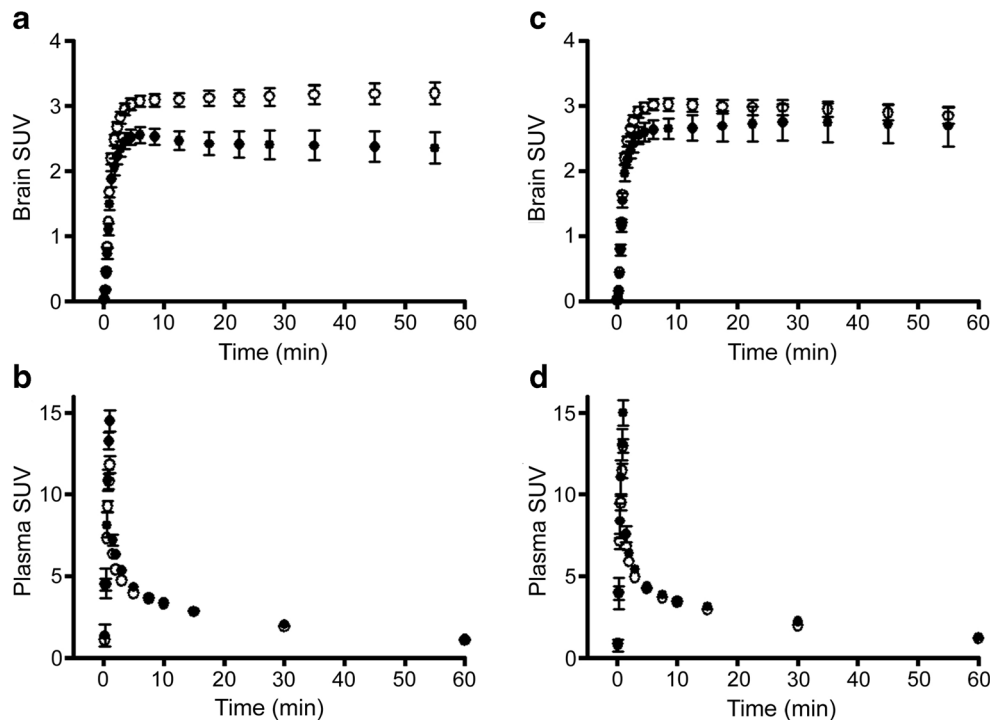


Fig. 2. Time-activity curves for [^{18}F]FDG uptake in **a** whole brain and **b** plasma at scan 1 and idem at scan 2 (**c** and **d**, respectively). Open symbols indicate data for female rats, whereas closed symbols concern males. Data plotted as mean \pm SEM.

proestrous and metestrous at scan 2, which was made after an interval of 9 to 13 days (in most cases, 10 days). However, due to variability of the cycle length, some females were not in proestrous or metestrous at retest. Two

were in proestrous and six were in metestrous, but two others were in estrous (sexually receptive, rising estrogen level) and six in diestrous (sexually quiescent, low estrogen level) by the time of the second scan (Table 1).

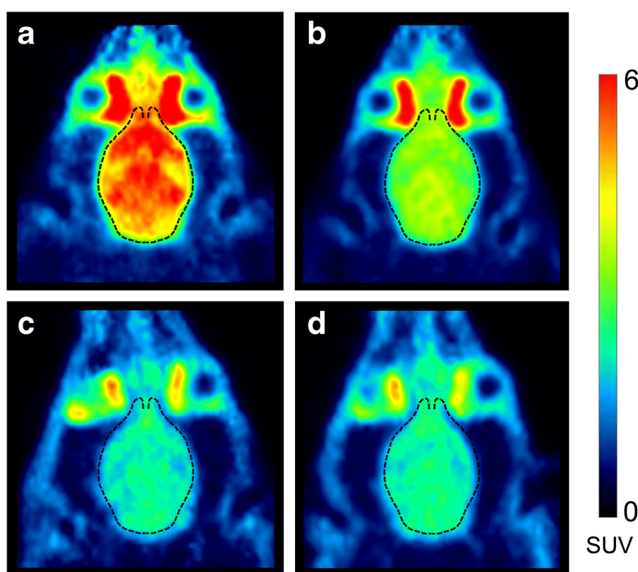


Fig. 3. Coronal [^{18}F]FDG-PET images of the brain of (**a**, **b**) a female and (**c**, **d**) male rat (test, retest). The images represent data summed from 5 to 60 min after injection, and the outline of the brain is indicated by a dotted line.

Test-Retest Stability

Relative differences between test and retest besides TRV, COV, and ICC values for the parameters SUV, normalized SUV, glucose flux, and $\text{rCMR}_{\text{glucose}}$ are reported in the ESM Tables and in Fig. 6. These data indicate a greater relative difference between the scans and a greater TRV in females than in males. The most dramatic difference between the sexes was noted when ICC was calculated. Whereas male rats showed good to excellent values for the ICC of whole brain SUV (0.96), normalized SUV (0.92), flux (0.93), and rCMR (0.78), female rats showed moderate to poor ICC values for the same parameters (0.60, 0.61, 0.40, and 0.23, respectively). Similar gender differences were noted in all studied brain areas. Thus, the reproducibility of measurements of cerebral glucose metabolism was considerably better in males than in females.

Discussion

In brain tissue of isoflurane-anesthetized male rats and mice, metabolic rates of 23 ± 3 and 24 ± 6 μmol glucose/min/100 g

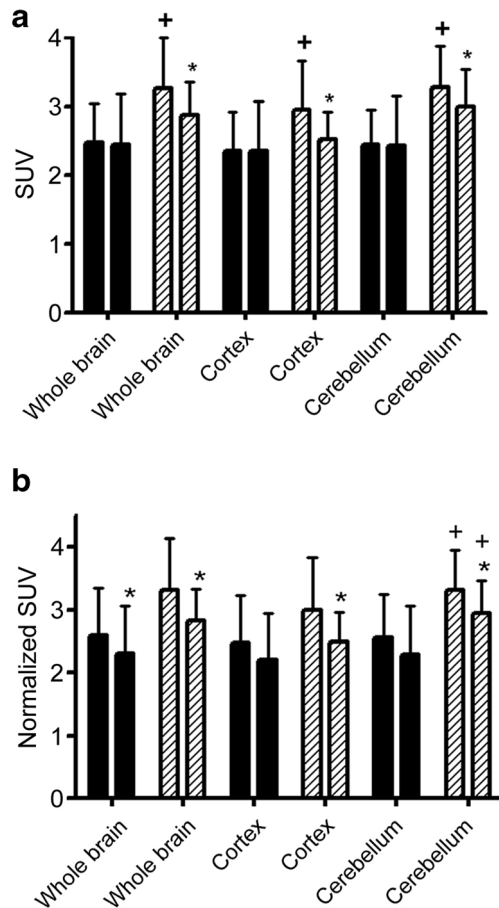


Fig. 4. **a** SUV and **b** normalized SUV of [^{18}F]FDG in whole brain, cortex, and cerebellum at test and retest. Solid bars indicate data of male, and hatched bars data of female rats, plotted as mean \pm SD. * = Significantly different from the test value; + = significantly different from the corresponding value in males.

have been reported [26, 27]. Our estimate of $20.8 \pm 1.4 \mu\text{mol}/\text{min}/100 \text{ g}$ at the first scan corresponds closely to these literature values. Published test-retest studies for [^{18}F]FDG-PET in rat brain involved a small number of conscious animals and concerned only males. In these studies, $\text{rCMR}_{\text{glucose}}$ was reported as $99.6 \pm 10.2 \mu\text{mol}/\text{min}/100 \text{ g}$ [14] and relative differences between retest and test in various brain regions ranged from -7 to $+10 \%$ [13]. Our measurements of cerebral $\text{rCMR}_{\text{glucose}}$ in anesthetized male (19.1 ± 2.0) and female (18.3 ± 0.6) rats showed similar standard deviations (maximally 10 % of the mean), but we observed greater differences between retest and test, ranging from -12 to -22% . This last observation may be due to the fact that we included animals from both sexes, and female rats showed greater differences between retest and test than males (Fig. 4a).

Although we did not observe any significant difference in cerebral $\text{rCMR}_{\text{glucose}}$ of male and female rats or in $\text{rCMR}_{\text{glucose}}$ of female rats at proestrous and metestrous, the sexes showed different values for SUV,

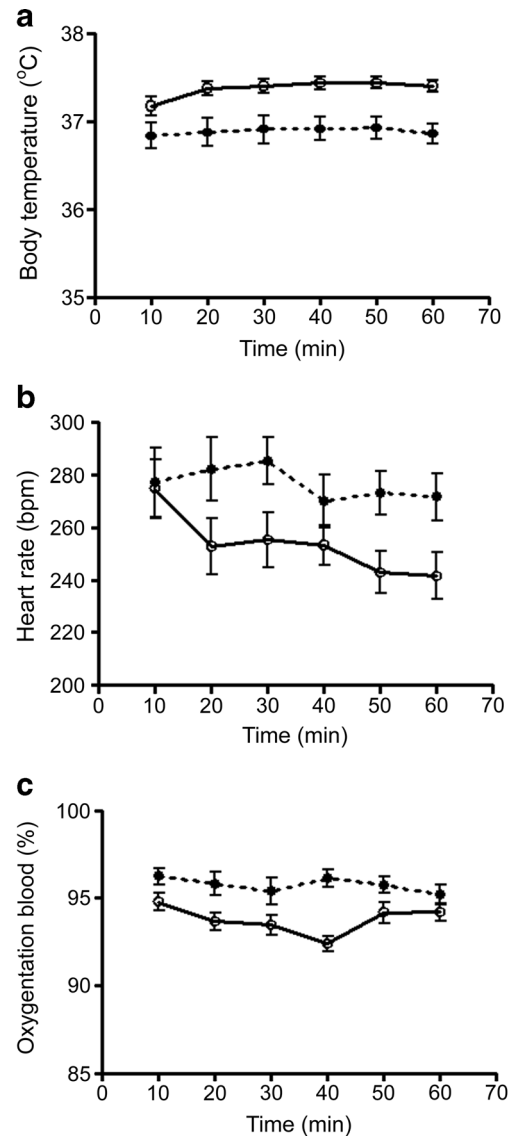


Fig. 5. Time course of: **a** rectal temperatures, **b** heart rate, and **c** oxygenation of the blood during the PET scans. Open symbols indicate data for female rats, whereas closed symbols concern males. Data plotted as mean \pm SEM.

normalized SUV, and glucose influx. We noted higher values for these parameters in females, as was reported previously in some studies of [^{18}F]FDG uptake in the human brain [1–3]. Moreover, these parameters showed higher values at test than at retest. If we quantified regional rather than whole brain SUV or glucose influx, similar differences between the sexes or scans were noted in all studied brain regions. This global character of the observed phenomena suggests that tracer input was different in males and females, and at retest and test. Since $\text{rCMR}_{\text{glucose}}$ did not show any differences, the detected differences of SUV, normalized SUV, and glucose flux appear to be related to altered levels of non-radioactive glucose in blood plasma.

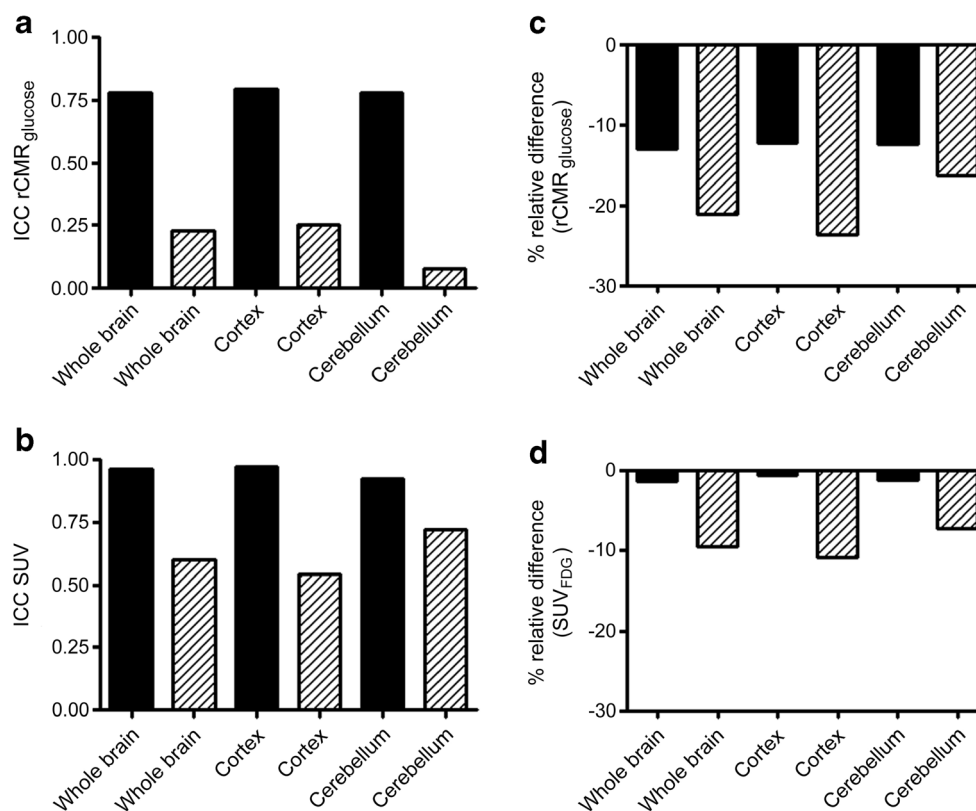


Fig. 6. **a, b** ICC values and **c, d** relative difference between retest and test for rCMR_{glucose} and SUV in whole brain, cortex, and cerebellum. Solid bars indicate data of male, and hatched bars data of female rats.

The physiological data that we recorded during our scans indicate that male and female rodents respond differently to the conditions which are encountered in small animal PET. Body temperature, heart rate, and blood oxygenation were more stable in males than in females during prolonged isoflurane anesthesia, although the observed differences between the sexes were small. Blood glucose levels during the scan were also different, namely higher in males than in females.

The relative difference between the scans and the TRV of rCMR_{glucose} and all other calculated parameters of cerebral glucose metabolism were greater in females than in males. Moreover, the ICC of these parameters was good to excellent in males but poor to moderate in females. The test-retest stability of [¹⁸F]FDG-PET was thus considerably greater in male than in female rats.

Conclusion

This study has several limitations: It involved only a single tracer, a single form of anesthesia, a single rodent species, and animals at a single age. Thus, our findings cannot be generalized, particularly not to other tracers, other biochemical processes or awake rats. However, our findings support the hypothesis that female rats show greater variability than

male rats in small animal imaging. Based on these results for [¹⁸F]FDG in anesthetized rats, the mixing of sexes in rodent imaging will result in an impaired test-retest stability of PET data and a need for larger group sizes to maintain the same statistical power in group comparisons.

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Compliance with Ethical Standards. The study protocol complied to European Directive 2010/63/EU and the Law on Animal Experiments of The Netherlands; it was approved by the Central Committee on Animal Experiments of The Netherlands (The Hague, license no. AVD105002015166) and the Institutional Animal Care and Use Committee of the University of Groningen (protocol 15166-01-001).

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

- Baxter LR Jr, Mazziotta JC, Phelps ME, Selin CE, Guze BH, Fairbanks L (1987) Cerebral glucose metabolic rates in normal human females versus normal males. *Psychiatry Res* 21:237–245
- Andreason PJ, Zametkin AJ, Guo AC, Baldwin P, Cohen RM (1994) Gender-related differences in regional cerebral glucose metabolism in normal volunteers. *Psychiatry Res* 51:175–183
- Yoshizawa H, Gazes Y, Stern Y, Miyata Y, Uchiyama S (2014) Characterizing the normative profile of 18F-FDG PET brain imaging: sex difference, aging effect, and cognitive reserve. *Psychiatry Res* 221:78–85
- Hebert JR, Kindred JH, Bucci M, Tuulari JJ, Brenner LA, Forster JE, Koo PJ, Rudroff T (2016) Cognitive-motor related brain activity during walking: differences between men and women with multiple sclerosis. *Arch Phys Med Rehabil* 97:61–66
- Tyler JL, Strother SC, Zatorre RJ, Zatorre RJ, Alivisatos B, Worsley KJ, Diksic M, Yamamoto YL (1988) Stability of regional cerebral glucose metabolism in the normal brain measured by positron emission tomography. *J Nucl Med* 29:631–642
- Miura SA, Schapiro MB, Grady CL, Kumar A, Salerno JA, Kozachuk WE, Wagner E, Rapoport SI, Horwitz B (1990) Effect of gender on glucose utilization rates in healthy humans: a positron emission tomography study. *J Neurosci Res* 27:500–504
- Azari NP, Rapoport SI, Grady CL, DeCarli C, Haxby JV, Schapiro MB, Horwitz B (1992) Gender differences in correlations of cerebral glucose metabolic rates in young normal adults. *Brain Res* 574:198–208
- Volkow ND, Wang GJ, Fowler JS, Hitzemann R, Pappas N, Pascani K, Wong C (1997) Gender differences in cerebellar metabolism: test-retest reproducibility. *Am J Psychiatry* 154:119–121
- Gur RC, Mozley LH, Mozley PD, Resnick S, Karp J, Alavi A, Arnold S, Gur R (1995) Sex differences in regional cerebral glucose metabolism during a resting state. *Science* 267:528–531
- Hu Y, Xu Q, Li K, Zhu H, Qi R, Zhang Z, Lu G (2013) Gender differences of brain glucose metabolic networks revealed by FDG-PET: evidence from a large cohort of 400 young adults. *PLoS One* 8:e83821
- Kawachi T, Ishii K, Sakamoto S, Matsui M, Mori T, Sasaki M (2002) Gender differences in cerebral glucose metabolism: a PET study. *J Neurol Sci* 199:79–83
- Hsieh TC, Lin WY, Ding HJ, Sun SS, Wu YC, Yen KY, Kao CH (2012) Sex- and age-related differences in brain FDG metabolism of healthy adults: an SPM analysis. *J Neuroimaging* 22:21–27
- Moore AH, Osteen CL, Chatziioannou AF, Hovda DA, Cherry SR (2000) Quantitative assessment of longitudinal metabolic changes in vivo after traumatic brain injury in the adult rat using FDG-microPET. *J Cereb Blood Flow Metab* 20:1492–1501
- Huang CC, Wu CH, Huang YY, Tzen KY, Chen SF, Tsai ML, Wu HM (2017) Performing repeated quantitative small-animal PET with an arterial input function is routinely feasible in rats. *J Nucl Med* 58:611–616
- Murphy DG, DeCarli C, McIntosh AR, Daly E, Mentis MJ, Pietrini P, Szczepanik J, Schapiro MB, Grady CL, Horwitz B, Rapoport SI (1996) Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. *Arch Gen Psychiatry* 53:585–594
- Hamacher K, Coenen HH, Stocklin G (1986) Efficient stereospecific synthesis of no-carrier-added 2-[¹⁸F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *J Nucl Med* 27:235–238
- Hubscher CH, Brooks DL, Johnson JR (2005) A quantitative method for assessing stages of the rat estrous cycle. *Biotech Histochem* 80:79–87
- Sijbesma JW, Zhou X, Vázquez García D et al (2016) Novel approach to repeated arterial blood sampling in small animal PET: application in a test-retest study with the adenosine A1 receptor ligand [¹¹C]MPDX. *Mol Imaging Biol* 18:715–723
- Vázquez García D, Casteels C, Schwarz AJ et al (2015) A standardized method for the construction of tracer specific PET and SPECT rat brain templates: validation and implementation of a toolbox. *PLoS One* 10:e0122363
- Schwarz AJ, Danckaert A, Reese T, Gozzi A, Paxinos G, Watson C, Merlo-Pich EV, Bifone A (2006) A stereotaxic MRI template set for the rat brain with tissue class distribution maps and co-registered anatomical atlas: application to pharmacological MRI. *Neuroimage* 32:538–550
- Patlak CS, Blasberg RG (1985) Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. *J Cereb Blood Flow Metab* 5:584–590
- Patlak CS, Blasberg RG, Fenstermacher JD (1983) Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 3:1–7
- Julien-Dolbec C, Tropes I, Montigon O, Reutenauer H, Ziegler A, Decorps M, Payen JF (2002) Regional response of cerebral blood volume to graded hypoxic hypoxia in rat brain. *Br J Anaesth* 89:287–293
- Seibyl JP, Marek K, Sheff K, Baldwin RM, Zoghbi S, Zea-Ponce Y, Charney DS, van Dyck C, Hoffer PB, Innis RB (1997) Test/retest reproducibility of iodine-123-betaCIT SPECT brain measurement of dopamine transporters in Parkinson's patients. *J Nucl Med* 38:1453–1459
- Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics* 33:159–174
- Lenz C, Rebel A, van Ackern K, Kuschinsky W, Waschke KF (1998) Local cerebral blood flow, local cerebral glucose utilization, and flow-metabolism coupling during sevoflurane versus isoflurane anesthesia in rats. *Anesthesiology* 89:1480–1488
- Toyama H, Ichise M, Liow JS, Modell KJ, Vines DC, Esaki T, Cook M, Seidel J, Sokoloff L, Green MV, Innis RB (2004) Absolute quantification of regional cerebral glucose utilization in mice by ¹⁸F-FDG small animal PET scanning and 2-¹⁴C-DG autoradiography. *J Nucl Med* 45:1398–1405